AR201-13459

American Chemistry Council *Good Chemistry Makes It Possible*

December 21, 2001

Via Certified Mail and e-mail

Christine Todd Whitman, Administrator
U.S. Environmental Protection Agency (EPA)
P.O. Box 1473
Merrifield, VA 22116

Re: **Brominated Flame Retardant Industry Panel (BFRIP),
HPV Chemical Challenge Program Submission,
Test Plans and Data for Cyclododecane, 1,2,5,6,9,10-hexabromo- (CAS No. 319-455-6) and Phenol, 4,4-isopropylidenebisO2,6-dibromo-(sic) (CAS No. 79-94-7)**

Dear Administrator Whitman:

The BFRIP of the American Chemistry Council is pleased to submit the attached data assessment for Cyclododecane, 1,2,5,6,9,10-hexabromo- (CAS No. 319-455-6) and Phenol, 4,4-isopropylidenebisO2,6-dibromo-(sic) (CAS No. 79-94-7) to EPA's HPV Chemical Challenge Program (Program). This submission fulfills BFRIP's commitment to the Program for the year 2001. Data for two additional chemicals will be submitted in time to meet our commitment for 2003. BFRIP member companies are Albemarle Corp., Great Lakes Chemical Corp. and Ameribrom, Inc., a subsidiary of Bromine Compounds Ltd.

In addition to the test plans and data summaries for (CAS No. 319-455-6) and (CAS No. 79-94-7), please also find a set of robust summaries contained in EPA's HPV format document for both of these chemicals.

This submission is also being sent electronically to the following e-mail addresses:

Oppt.ncic@epa.gov
Chem.rtk@epa.gov

In preparing this test plan, the Panel has given careful consideration to the principles contained in the letter EPA sent to all Program participants on October 14, 1999. As requested by EPA in that letter, the Panel has sought to maximize the use of scientifically appropriate categories of related chemicals and of structure activity relationships.

MR-53600

If you require additional information, please contact the BFRIP's technical contact, Wendy K. Sherman at (703) 741-5639 or wendy_sherman@americanchemistry.com.

Sincerely yours,

Courtney M. Price
Vice President, CHEMSTAR

Attachments

cc: C. Auer, EPA/OPPT
B. Leczynski, EPA/OPPT
BFRIP Members
Steve Russell, ACC (without attachments)

AR201-13459A

HPV

DATA SUMMARY AND TEST PLAN

FOR

HEXABROMOCYCLODODECANE (HBCD)

CAS No. 3194556

Prepared by

American Chemistry Council
Brominated Flame Retardant Industry Panel (BFRIP)
1300 Wilson Blvd
Arlington,VA

December 20, 2001

1.0 INTRODUCTION

The Brominated Flame Retardant Industry Panel (BFRIP) was formed in the 1980s to address issues related to the brominated flame retardants that its members manufacture in common, conduct research, and interact with regulatory agencies and other interested parties. Its members, who are global manufacturers of brominated flame retardants, are Albemarle Corporation, Ameribrom Inc. (a subsidiary of Dead Sea Bromine Group), and Great Lakes Chemical Corporation. Akzo-Nobel is an associate member. BFRIP, organized under the American Chemistry Council, volunteered under the U.S. EPA's High Production Volume (HPV) program to prepare the Data Summary/Test Plan and Robust Summaries for hexabromocyclododecane (HBCD). As discussed below, HBCD is a data-rich chemical, including valid studies or other information on all SIDS endpoints. For this reason, no additional tests are proposed for the purpose of this program.

2.0 HBCD's STRUCTURE AND PROPERTIES

HBCD, a solid at room temperature, is a cyclic aliphatic flame retardant (Fig. 1) with a molecular weight of 641.7. The commercial product is a mixture of three stereoisomers, alpha, beta and gamma, which are typically present at approximately 6, 8, and 80%, respectively.

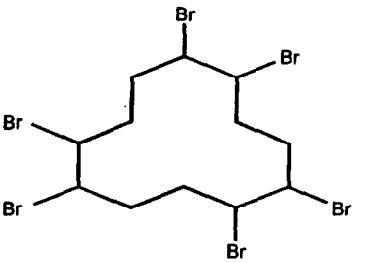

Figure 1. Hexabromocyclododecane (HBCD).

The measured physical/chemical properties of the commercial HBCD product are as follows: water solubility 3.4 ug/L at 25°C *(Stenzel, J. and Markley, B. 1997)*, vapor pressure 6.27 x 10^{-5} Pa at 21°C *(Stenzel, J and Nixon, W. 1997)*, and log octanol-water partition coefficient 5.625 at 25°C *(MacGregor, J and Nixon, W. 1997)*. The test article used for these measurements was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc., and the studies were conducted according to EPA, OECD and GLP guidelines. The product's melting point is ~ 186°C (range: 175-195°C) *(Albemarle, 2001)*.

3.0 HBCD APPLICATIONS

HBCD is used as a flame retardant. Its primary application is in extruded (XPS) and expanded (EPS) polystyrene foam that is used as thermal insulation in the building

industry. HBCD is highly efficient in this application so that very low levels are required to reach the desired flame retardancy. Typical HBCD levels in EPS are 0.67% and in XPS 2.5%. At present, HBCD is the only suitable flame retardant for these applications. Any other flame retardant would likely need higher load levels in the polystyrene foam.

A secondary, though important, application of HBCD is as a flame retardant for upholstery textiles. In this application, HBCD is applied to the back of the upholstery fabric encapsulated in a polymer. Typical HBCD levels in the polymer backcoat are 6-15%. The potential exposure and hazard to consumers associated with this use were reviewed recently by the U.S. National Research Council *(D. Gardner and B. Walker, Chair, Toxicological Risks of Selected Flame Retardants, 2000, National Academy Press, Washington, D.C.; **http://www.nap.edu**)*. The review found that direct exposure to the consumer was likely to be minimal, that the hazard index was less than 1 for all exposure routes (e.g. not likely to pose a health hazard), and that no further research was needed for assessing health risks from HBCD.

A very minor application for HBCD is in video or audio equipment housings where V-2 levels of flame retardancy are acceptable. HBCD is not used to flame retard electronic housings (e.g. television sets) that must meet the higher V-0 flame retardancy standard.

4.0 HBCD TOXICOLOGY DATA SUMMARY

4.1 ENVIRONMENTAL FATE (BFRIP)

HBCD's measured and predicted environmental fate parameters are provided in Table 1.

HBCD is predicted to partition in the environment to soil and sediment (~98%) where it will bind extensively to organic carbon (estimated Koc_{soil} = 1.25 x 10^5) and to be essentially immobile in soil. Based on a release of 1,000 kg/hr to air, water and soil, the predicted partitioning is: air 0.0007%, water 2.1%, soil 40% and sediment 58% *(Level III Fugacity Model, EPIWIN V3.04, Syracuse Research Corporation)*. HBCD is not expected to volatilize from water based on its river and lake volatilization half-lives and air-water partition coefficient. HBCD is expected to partition from water to organic matter (biomass to water partition coefficient = 1 x 10^7) *(EPIWIN V3.04, Syracuse Research Corporation)*. Sewage treatment plants are predicted to remove HBCD from the influent to a high degree (94% removal), but biodegradation in the treatment plant is not expected. Removal in treatment plants is via partitioning to sludge.

4.1.1 Photodegradation

No photodegradation study has been performed on HBCD. However, in the event HBCD were able to undergo photodegradation, this is not expected to be a significant route of environmental degradation due to its low vapor pressure (6.27 x 10^{-5} Pa at 21°C) that would preclude substantial levels in the air.

4.1.2 Stability in Water (Hydrolysis)

HBCD is not expected to undergo hydrolysis. In the event HBCD were subject to hydrolysis, this is not expected to be a significant route of environmental degradation due to its low water solubility (3.4 ug/L).

4.1.3 Biodegradation: Closed Bottle Test For Biodegradability (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD guidelines and Good Laboratory Practices.

HBCD was tested for ready biodegradation in a 28 day closed bottle test at a concentration of 7.7 mg/L by measuring dissolved oxygen uptake and expressing it as a percentage of the theoretical oxygen demand or chemical oxygen demand. No biodegradation was observed; the percent biodegradation was 0 (*Schaefer, E and Haberlein, D., 1996, Hexabromocyclododecane (HBCD): Closed Bottle Test. Project No.: 439E-102. Wildlife International Ltd. Easton, MD).*

4.1.2 Transport (Fugacity) (BFRIP)

If released in equal amounts to air, water and soil, HBCD was predicted to partition to soil and sediment. Based on a release of 1,000 kg/hr to air, water and soil, the predicted partitioning would be: air – 0.0007%, water - 2.1%, soil - 40%, and sediment - 58%. The majority (86%) would be reacted in sediment (63%) and soil (23%) with only 11% of the total undergoing advection (*Level III Fugacity Model, EPIWIN modeling software, V3.04, Syracuse Research Corporation).*

4.2 ECOTOXICOLOGY DATA

HBCD was not acutely toxic to fish, daphnia or freshwater or marine alga at the limits of its water solubility. HBCD was not chronically toxic to daphnia nor was it toxic to fish early life stages at the limits of its water solubility. HBCD was bioconcentrated in fish.

4.2.1 Acute Toxcity to Fish

4.2.1.1 96-Hour Acute Toxicity Test With Rainbow Trout *(Oncorhynchus mykiss)* (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD guidelines and Good Laboratory Practices.

HBCD was not acutely toxic to rainbow trout. HBCD's 96 hour LC50, no mortality concentration and no observed effect concentration were all > than HBCD's water solubility. The highest nominal dose tested was twice HBCD's water solubility.

TABLE 1. Environmental Fate Parameters for HBCD.

Parameter	Estimation Program or Test Result	Result
Photodegradation	-	Not likely to be a significant route of environmental degradation due to low vapor pressure
Hydrolysis	-	Not likely to be a significant route of environmental degradation due to low water solubility
Transport	Calculated (EPIWIN QSAR; EUSES)	Atmospheric half life = 1.75 day Subcooled vapor pressure = 4.93 x 10-3 Pa
Distribution	Estimated (EPI win, V.3.04)	Level III Fugacity Model predicts at 1000 kg/Hr emissions to air, water and soil: Air 0.0007%, Water 2.1%, Soil 40%, Sediment 58%
Atmospheric Oxidation	Estimated (EPI win, V.3.04)	Overall OH Rate Constant = 5.0 x 10-12 cm3/molecule-sec Half-Life = 2.1 Days (12-hr day; 1.56 x 10+6 OH/cm3) Half-Life = 25.6 Hrs
Henry's Law Constant	Estimated (EPI win, V.3.04)	6.4 x 10-11 atm-m3/mole at 25 °C 2.6 x 10-9 unitless at 25 °C
Soil Koc	Estimated (EPI win, V.3.04)	1.25 x 10+5
Octanol-Water Partition Coefficient	Estimated (EPI win, V.3.04)	5.4 x 10+7
Air-Water Partition coefficient	Estimated (EPI win, V.3.04)	2.6 x 10+7
Biomass to Water Partition Coefficient	Estimated (EPI win, V.3.04)	1.1 x 10+7
Volatization from Water	Estimated (EPI win, V.3.04)	Half life: 2,631 years (River); 2.8x10+4 years (Lake)
Sewage Treatment Plant Fugacity Model	Estimated (EPI win, V.3.04)	Total Removal: 94%, Total Biodegradation: 0.78%, Primary Sludge: 59.87%, Waste Sludge: 33.35%, Final Water Effluent: 6%
Level III Fugacity Model	Estimated (EPI win, V.3.04)	At Emissions to Air, Water, Soil and Sediment of 1,000, 1,000, 1,000 and 0 kg/hr, respectively: Fugacity (atm): Air 9.9 x 10-15, Water 2.7 x 10-18, Soil 4.1 x 10-20, Sediment 2.6 x 10–18 Reaction (kg/hr): Air 0.91, Water 97.7, Soil 1.9 x10+3, Sediment 686 Advection (kg/hr): Air 0.67, Water 203, Soil 0, Sediment 114 Reaction (%): Air 0.03, Water 3.3, Soil 63.3, Sediment 22.9 Advection (%): Air 0.02, Water 6.8, Soil 0, Sediment 3.8
Biodegradation	OECD, GLP (CMA BFRIP 1996)	Not readily biodegradable

Nominal test concentrations were 0, 1.5, 2.2, 3.2, 4.6 and 6.8 ug/L and corresponded to

mean measured concentrations (HPLC with UV/VIS detector) of 0, 0.75, 1.5, 2.3, 2.3 and 2.5 ug/L, respectively *(Graves, W and Swigert, J. (1997) Hexabromocyclododecane (HBCD): A 96-Hour Flow-Through Acute Toxicity Test with the Rainbow Trout (Oncorhynchus mykiss). Project Number: 439A-101. Wildlife International LTD, Easton, MD).*

4.2.1.2 Other Studies

The lack of acute toxicity in rainbow trout at HBCD's limit of water solubility is consistent with earlier studies performed at substantially higher concentrations. A Velsicol study in 1975 reported that the LC50 (96 Hr) in Bluegill sunfish (*L. macrochirus*) was >100 mg/L (nominal). A BASF study reported that the 96 hr LC50 in Golden orf (*L. idus*) was >10,000 mg/L (nominal).

4.2.2 Acute Toxicity to Aquatic Invertebrates: 48-Hour Acute Toxicity Test With *Daphnia magna* (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD guidelines and Good Laboratory Practices.

HBCD was not acutely toxic to *Daphnia magna.* HBCD's 48 hour EC50, no mortality/immobility concentration, and no observed effect concentration (6.8 ug/L nominal) in *Daphnia magna* were all > than HBCD's water solubility (3.4ug/L measured). The highest nominal dose tested was twice HBCD's water solubility. Nominal test concentrations were 0, 1.5, 2.2, 3.2, 4.6 and 6.8 ug/L which corresponded to mean measured concentrations (HPLC with UV/VIS detector) of 0, 2.4, 1.8, 2.1, 2.3 and 3.2 ug/L, respectively *(Graves, W and Swigert, J. (1997) Hexabromocyclododecane (HBCD): a 48-hour flow-through acute toxicity test with the cladoceren (Daphnia magna). Project Number: 439A-102. Wildlife International Ltd., Easton, MD).*

4.2.3 Toxicity to Aquatic Plants

4.2.3.1 96-Hour Acute Toxicity Test With The Freshwater Alga *(Selenastrum capricornutum)* (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD guidelines and Good Laboratory Practices. This study was performed to complete the EU base set.

HBCD was not acutely toxic to *Selenastrum capricornutum.* HBCD's 96 hour EC10, EC50, EC90 and no observed effect concentration were all > than HBCD's water solubility. The highest nominal dose tested was twice HBCD's water solubility. Dose levels were 0, 1.5, 2.2, 3.12 4.6 and 6.8 ug/L (nominal). The mean measured concentration (HPLC with UV/VIS detector) at the 6.8 ug/L dose was 3.7 ug/L *(Roberts,*

C. and Swigert, J.. Hexabromocyclododecane (HBCD): A 96-Hour Toxicity Test with the Freashwater Alga (Selenastrum capricornutum). Wildlife International Ltd. Project Number: 439A-103. June 3, 1997. Wildlife International Ltd., Easton, MD).

4.2.4.3 Marine Alga

Walsh et al. 1987 *(Ecotoxicology and Environmental Safety, 14, 215-222)* reported testing the effect of media and test chemicals on acute toxicity in marine algae. HBCD was tested in 3 species of marine algae, and was not toxic at the limits of its water solubility. The EC50's are as follows: *Chlorella sp* 96 hr EC50 > water solubility (>1500ug/L); *S. costatum* 72 hr EC50 > water solubility (9.3-12 ug/L); *T. pseudonana* 72 hr EC50 > water solubility (50-370 ug/L).

4.2.5 Prolonged Exposure Data

HBCD was not toxic to fish early life stages or daphnia when exposed for prolonged periods of time. HBCD was bioconcentrated in fish.

4.2.5.1 Fish Early Life Stage In Rainbow Trout *(Oncorhynchus mykiss)* (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current OECD guidelines and Good Laboratory Practices.

Rainbow trout embryos were exposed to nominal HBCD water concentrations of 0.43, 0.85, 1.7, 3.4 and 6.8 ug/L. The top two doses represent HBCD's water solubility (3.4 ug/L) and two times HBCD's water solubility (6.8 ug/L). A negative control and solvent control group were also included. Mean measured concentrations (LC/MS with heated nebulizer operated in the selective ion monitoring mode) were 0.25, 0.47, 0.83, 1.8 and 3.7 ug/L. This method was designed to monitor for all 3 HBCD diastereomers; however, the trace residues of the alpha and beta diastereomers were evident in the water samples were below the established limits of quantitation. Comparison of the chromatograms from study initiation through study termination showed that the relative distribution of the HBCD diastereomers remained constant during the definitive study, and the gamma diastereomer measured results were consistent throughout the study.

Hatching success, time to hatch, time for larvae to swim-up, and post-hatch growth and survival were evaluated during the 88-day test. Rainbow trout exposed to HBCD at mean measured concentrations up to 3.7 ug/L (nominal concentration = 6.8 ug/L or twice HBCD's water solubility) for a 27-day hatching period and 61 days post-hatch showed no effects on hatching success, time to swim-up, larval survival, fry survival or growth. Consequently, HBCD was not chronically toxic to rainbow trout at concentrations at or above its limit of solubility. The NOEC for this study was 3.7 ug/L or 6.8 ug/L nominal (twice HBCD's water solubility). The low-effect-concentration (LOEC) and maximum acceptable toxicant concentration (MATC) could not be determined due to absence of

toxicity, but were considered >3.7 ug/L or >6.8 ug/L nominal (> twice HBCD's water solubility) (*Drottar et al. 2001. Hexabromocyclododecane (HBCD): An early life-stage toxicity test with the rainbow trout (Onchorhynchus mykiss). Project No.: 439A-112. Wildlife International, Ltd. Easton, MD).*

4.2.5.2 Flow Through Bioconcentration In Rainbow Trout *(Oncorhynchus mykiss)* (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD and GLP guidelines.

Nominal test concentrations were 0, 0.34, and 3.4 ug HBCD/L. These doses are equivalent to HBCD's water solubility and one tenth of its water solubility. Mean measured (LC/MS with heated nebulizer operated in the selected ion monitoring mode) test concentrations were 0, 0.18, and 1.8 ug HBCD/L. The length of the test was 70 days (35-day uptake, 35-day depuration). The steady bioconcentration factor (BCF) at a nominal concentration of 3.4 ug HBCD/L (1.8 ug HBCD/L measured) in whole fish was 8,974. This BCF was further defined as 4,650 in edible tissue and 12,866 in non-edible tissue. Steady state was not achieved at the nominal concentration of 0.34 ug HBCD/L due to an unexpected increase in tissue concentrations at day 35. The unexpected increase in tissue concentrations on day 35 may have been due to the variability in the measured water concentrations in this treatment group. The variability in turn is likely a function of the extremely low nominal concentration at this dose level (0.34 ug HBCD/L). Thus, the calculated BCF in the nominal 3.4 ug HBCD/L treatment group is considered a better estimate than that in the 0.34 ug HBCD/L treatment group *(Drottar, K. and Krueger, H. 2000. Hexabromocyclododecane (HBCD): Flow-through bioconcentration test with rainbow trout (Oncorhynchus mykiss). Project No.: 439A-111. Wildlife International, Ltd. Easton, MD).*

4.2.5.3 *Daphnia magna* Life Cycle (21 Day) (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD and GLP guidelines.

Nominal test concentrations were 0.85, 1.7, 3.4, 6.8 and 13.6 ug HBCD/L water; dose levels were based on HBCD's water solubility, 3.4 ug/L. Measured test concentrations (LC/MS with negative ion atmospheric pressure ionization) were 0.87, 1.6, 3.1, 5.6 and 11 ug HBCD/L water. No statistically significant effects on survival, reproduction or growth of *Daphnia magna* were seen at HBCD concentrations $\leq$ 3.1 ug/L (measured). Thus, HBCD's no effect concentration (NOEC), based on survival, reproduction and growth, to daphnia magna for 21 days was equivalent to HBCD's water solubility. The measured NOEC in this study was 3.1 ug/L and corresponded to a nominal HBCD concentration of 3.4 ug/L, e.g. HBCD's water solubility. The lowest observed effect concentration (LOEC) and the maximum acceptable toxicant concentration (MATC)

based on survival, growth and reproduction were greater than HBCD's water solubility. The LOEC, 5.6 ug/L, corresponded to nominal concentrations twice HBCD's water solubility. The effect seen at this dose level was a reduction in length. Survival and reproduction at the 5.6 ug/L dose level were not affected. The MATC, 4.2 ug/L, was calculated as the mean of the NOEC and the LOEC. The MATC was greater than HBCD's water solubility *(Drottar, K. and Krueger, H. 1998. Hexabromocyclododecane (HBCD): Flow-through life-cycle toxicity test with the cladocerna (Daphnia magna). Project No.: 439A-108. Wildlife International, Ltd. Easton, MD).*

4.3 MAMMALIAN TOXICOLOGY DATA

HBCD was not acutely toxic to rats on oral or dermal exposure. In repeated dose studies in rats (28 and 90-day studies), HBCD's no adverse effect level (NOAEL) was 1,000 mg/kg/day. HBCD did not induce developmental effects in the rat (NOAEL = 1,000 mg/kg/d). No evidence of carcinogenicity was found in an 18 month mouse study. HBCD did not induce mutations in the Ames, *in vitro* chromosome aberration, and *in vivo* mouse micronucleus tests.

4.3.1 Acute Mammalian Toxicology Data

HBCD was not acutely toxic to rats or rabbits during oral, dermal or inhalation exposure. The rat oral LD50 was >10 g/kg. The rabbit dermal LD50 was >8 g/kg. The rat inhalation LC50 was > 200 mg/L. HBCD was not irritating to the skin or eye when tested in rabbits. *(Lewis, C. 1978. Experiment Reference No. 78385-2 and 78385-1. Consumer Product Testing, Fairfield, NJ).*

4.3.2 Repeated Dose Toxicology Data

In repeated dose studies in rats, HBCD's no adverse effect level was at or near 1,000 mg/kg/day. Two 28-day studies and two 90-day studies have been performed.

4.3.2.1 Rat 28 Day Subchronic (BFRIP)

This study was conducted according to OECD and GLP guidelines. The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc.

HBCD, in the vehicle corn oil, was administered orally by gavage to three groups of Sprague-Dawley Crl: CD BR rats for a period of 28 consecutive days. Dose levels were 125 (low), 350 (mid), or 1,000 (high) mg/kg/day, administered at dosage volume of 5 ml/kg. The test groups consisted of 6 males and 6 females in the 125 and 350 mg/kg/day groups and 12 males and 12 females in the 1000 mg/kg/day group. A concurrent control group comprised of 12 males and 12 females received the vehicle, corn oil, for 28 consecutive days at a dosage volume of 5 ml/kg. At the end of the dosing period, 6 animals/sex/group were sacrificed and necropsied. The remaining 6 animals/sex in the

control and 1000 mg/kg/day groups remained on-test untreated for a 14-day recovery period. At the end of the recovery period, all animals were sacrificed and necropsied.

Animals were observed twice daily for mortality and moribundity. Clinical signs were recorded daily. Body weight and food consumption were measured weekly. Functional observational battery and motor activity evaluations were performed during weeks -1 (pretest), 3, and 5 (recovery). Samples for hematology and serum chemistry evaluations were collected at the primary (28 day) and recovery (42 day) sacrifices. Complete necropsies were performed on all rats. The brain, liver, kidney, heart, spleen, testes and epididymus or ovaries, adrenal glands, and thymus from all animals were weighed at each sacrifice. Approximately 40 tissues were collected and preserved at each necropsy from all animals. The following tissues were examined microscopically from the control and high dose animals: liver, kidney, heart, spleen, testes (males), prostate (males), seminal vesicles (males), epididymus (males), ovaries (females), adrenal glands, thymus, bone with marrow (sternebra), brain, stomach, cecum, duodenum, ileum, jejunum, lymph node, peripheral nerve (sciatic), spinal cord, lung, trachea, uterus (females), urinary bladder, and all gross lesions. The lungs, liver, kidneys, stomach, thyroid, gross lesions and target organs were examined in all dose levels.

Survival was not affected by administration of the test article. All animals survived to the scheduled sacrifice. Clinical signs observed during the study were nonspecific, low in incidence, non-dose-related and not considered related to test article.

Body weights, weight gain and food consumption of treated animals were compared statistically by sex and treatment day to their respective control groups ($p \leq 0.05$ or 0.01) and were not affected by treatment. No statistically significant differences in body weight between control and treated animals were detected with the exception of an increase in mean female body weight in the 350 mg/kg/day group during week 2 of treatment. Mean female body weight at that time point was 196 g versus 179 in the control group. No statistically significant differences in body weight gain between control and treated animals were detected with the exception of a decrease in mean male body weight gain in the 1,000 mg/kg/day recovery group during week 1 of recovery. Mean male body weight gain at that time point was 21 g versus 31 in the control group; mean male body weight was not statistically different from the control mean. No statistically significant differences in food consumption between control and treated animals were detected with the exception of an increase in mean female food consumption in the 350 mg/kg/day group during weeks -1, 1, and 2 of treatment. Mean female food consumption at that those time points were 18, 17 and 17 g versus16, 15 and 15 g in the control group, respectively.

Functional observation battery and motor activity results from treated animals were compared statistically by sex and treatment day to their respective control groups ($p \leq 0.05$). These parameters were not affected by treatment with the test article. No statistically significant differences were observed between treated and control animals at any time point.

No statistically significant differences between treated and control animals were found for hematology parameters with the exception of an increase in the mean activated partial thromboplastin time in the 1000 mg/kg/day males on week 4 and a decrease in the mean prothrombin time in the 1000 mg/kg/day females on week 4. These statistical differences were not of toxicological significance.

No toxicologically significant effects on serum chemistry values related to test article administration were observed at the 28-day primary and 42-day recovery sacrifice. Scattered instances of statistically significant differences between treated and control animals were detected for some serum chemistry parameters at the 28-day primary sacrifice. These scattered statistical differences were not considered toxicologically significant because the statistical differences occurred: in the absence of a dose response, in the absence of the accompanying clinical chemistry changes expected, in the opposite direction from what occurs in a toxic state, in a direction which is without physiologic significance, or due to potential interference with the laboratory method. No statistically significant differences in serum chemistry parameters were detected between groups at the 42-day recovery sacrifice.

No gross lesions that could be attributed to the test article were detected at either necropsy. Gross lesions were nonspecific, low in incidence, non-dose-related and considered incidental.

No microscopic lesions that could be attributed to the test article were detected on histopathologic exam. Microscopic changes were nonspecific, low in incidence, non-dose-related and considered incidental.

No statistical significant differences in organ weight or organ to body weight ratios were detected between control and treated animals with one exception. Absolute liver weights were statistically significantly increased with respect to control means at the 28-day sacrifice in males in the high dose and females in the mid and high dose. Liver to body weight ratios in mid and high dose males and low, mid and high dose females were statistically significantly increased at the 28-day sacrifice. At the recovery sacrifice, male absolute and liver to body weight ratio were statistically comparable to the control mean whereas female absolute liver weights and liver to body weight ratio were statistically significantly increased with respect to control mean. The difference in absolute liver weight between control and treated females was less pronounced at the end of the recovery period, indicating the increase in liver weight was reversible in females as well as males. In the absence of test article related histopathologic and serum chemistry changes, increases in liver weight are considered an adaptive, rather than a toxic response, are not uncommon in the rat, and are most likely the result of microsomal induction.

In conclusion, no systemic toxicity was observed at any dose level. Based on the results of this study, the NOAEL (No Observed Adverse Effect Level) of HBCD administered orally to male and female rats for 28 consecutive days was 1,000 mg/kg/day *(Chengelis,*

C. 1996 A 28-day repeated dose oral toxicity study of HBCD in rats. Study No. WIL-186004. WIL Research Laboratories, Inc. Ashland, OH).

4.3.2.2 Rat 90 Day Subchronic (BFRIP)

This study was conducted according to OECD and GLP guidelines. The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc.

The test article, a composite of three lots of commercial hexabromocyclododecane (HBCD), was administered by oral gavage in corn oil once daily to four groups of Crl:CD(SD)IGS BR rats (n=15/sex/group) at dose levels of 0 (control), 100 (low), 300 (mid) and 1000 (high) mg/kg/day seven days per week for 90 days. The dosage volume was 5 ml/kg. The control animals received the vehicle, corn oil, only. At the end of the 90-day treatment period, 10 animals/sex/group were euthanized and necropsied. The remaining rats continued on test untreated for a 28-day recovery period prior to necropsy.

In addition to the main toxicology groups, two satellite groups of 20 animals/sex/group were treated concurrently in an identical manner at dose levels of 0 or 1000 mg HBCD/kg/day for up to 90 days. Body weights were recorded weekly. Two animals/sex/group were euthanized on study days 2, 6, 9, 13, 20, 27, 55, 89, 104 and 118, and blood and body fat (mesenteric and/or omental) were collected. The body fat was analyzed for HBCD content.

Animals in the main toxicology groups were observed twice daily throughout the study for mortality and morbidity. Body weights and food consumption were measured weekly. Blood was collected at study weeks 3 (n=5/sex/group), 13 (n=10/sex/group) and 17 (n=5/sex/group) for hematology, serum chemistry and hormone (T_3, T_4 and TSH) measurements. Urine was collected prior to each necropsy, at study weeks 13 and 17, for urinalysis. Ocular examinations were performed prior to study initiation and during study weeks 12 and 15. Functional Observational Battery and Locomotor Activity evaluations were performed on 5 animals/sex/group prior to study initiation, during the last week of test article administration (study week 13), and during the recovery period. An examination of vaginal cytology (for estrus cycle determinations) was performed on study days 69-90. At each necropsy, sperm motility/viability, morphology, and number were assessed. Complete necropsies were performed on all animals. Approximately 40 organs or tissues/animal were collected and preserved. The adrenals, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus, thyroids with parathyroids, and uterus with cervix were weighed. Paraffin sections of tissues stained with hematoxylin and eosin from the control and 1000 mg/kg/day dose groups and the liver, lungs and thyroid glands in the 100 and 300 mg/kg/day doses, and gross lesions from all animals were examined under the light microscope. Livers from five randomly chosen animals/sex from the control and 1000 mg/kg/day dose groups were examined microscopically using Oil Red O or periodic acid Schiff's (PAS) reagent for evidence of lipid accumulation or glycogen accumulation/depletion, respectively. Statistical

comparisons by sex and treatment day were made between the control and treated animals where indicated ($p<0.05$).

No test article-related effect on mortality occurred. Clinical signs were non-specific, low in incidence, non-dose-related and not related to test article administration. No test article-related changes occurred in body weight, food consumption, Functional Observational Battery or Locomotor Activity. No test article-related effects on hematologic parameters were noted. No test article-related ocular lesions were detected at the ophthalmic exams. No test article-related changes were noted on the estrus cycle as determined by vaginal cytology, or on sperm motility/viability, morphology, and number. Instances of statistically significant differences between control and some treatment groups were detected at study week 13 in the clinical chemistry data, hormone data, organ weight data and histology findings. They were generally secondary to the inducing effects on the liver or were otherwise not considered adverse effects of treatment as discussed further below.

Statistically significant ($p<0.05$) test article-related clinical chemistry changes at week 13 include an increase in albumin (all dose levels for males), total protein (all dose levels for females and 1000 mg/kg/day for males), globulin (300 and 1000 mg/kg/day for females), and chloride (all doses for both sexes). In addition, increased gamma glutamyltransferase levels were noted in the 1000 mg/kg/day group ($p<0.05$). Thyroxine (T_4) levels were decreased at study week 13 compared to the control mean in all male dose groups and the 300 and 1000 mg/kg/day dose females ($p<0.05$). There were no corresponding statistical effects on T_3 and TSH. While potentially test article-related, the changes in serum chemistry parameters were not of sufficient magnitude to be adverse, occurred in otherwise clinically normal animals, tended to be within or close to historical control values, and were not present at the end of the recovery period; furthermore, these serum albumin and gamma glutamyltransferase increases were probably secondary to the increases in liver weight. The increases in serum chloride were probably secondary to be presence of free bromide in the test article preparation which interfered with the chloride determination methodology. The decrease in T_4, which was also reversible, was also probably secondary to increased liver weight (secondary to microsomal enzyme induction, known to cause increased metabolism and clearance of T_4 in the rat).

The incidence of observations noted at gross necropsy was low and there was no evidence of frank organ damage. On histopathologic examination of tissues, relatively mild findings occurred in both the control and treated groups. Potential test article-related histologic changes were identified in the liver and thyroid glands but these would not be considered indicative of frank toxicity. These organs were examined microscopically in all groups at both necropsies. The liver changes in male rats at the 90-day necropsy (Study Week 13) were characterized as minimal hepatocellular vacuolation and occurred in 10% of control males and ~50% of the males at 100, 300 and 1000 mg/kg/day. Minimal hepatocellular vacuolation was also detected in females in the control and test article treated groups without a clear dose response (3 to 4/10 animals per group) but, mild and moderate vacuolation was detected in females only in the 300 (1/10) and 1000 mg/kg/day (2/10) dose groups. Minimal to mild hepatocellular hypertrophy

was also detected only in the 1000 mg/kg/day group (5/10) females. Minimal thyroid follicular cell hypertrophy was detected 1/10, 1/10, 5/10 and 7/10 males in the control, 100, 300 and 1000 mg/kg/day groups, respectively and in 4/10 and 3/10 females in the 300 and 1000 mg/kg/day groups respectively. In addition, mild thyroid follicular hypertrophy was detected in 4/10 females in the 1000 mg/kg/day group. The histologic changes in the liver were accompanied by an increase in liver weight. In contrast there were no statistically significant changes in thyroid weight (absolute, relative to body weight and relative to brain weight). At study week 13, mean liver weights in all dose levels of both sexes (absolute, relative to body weight and relative to brain weight) were increased compared to the male and female control means ($p<0.05$). The increases in liver weight were a result of a microsomal enzyme inducing effect and were not typically considered indicative of toxicity in absence of frank organ damage. The reversible histologic changes (vacuolation and hypertrophy) are often found to accompany increased liver weight caused by liver enzyme induction. At week 17, the liver changes (weight and histology) had at least partially, if not fully, resolved in all treated groups without delayed or long-term toxic effects. The histologic changes in the thyroid had also nearly completely resolved except in the 1000 mg/kg/day group females, where partial recovery occurred.

Increases in mean prostate weight were noted in the 1000 mg/kg/day group males at the primary necropsy. However, the increases in prostate weight were probably not of toxicological significance since the increases did not persist to the recovery period, there were no correlating histologic findings and no change in sperm production.

HBCD was detected in the adipose tissue of male and female rats treated with 1000 mg/kg/day for up to 90 days. Isomer-specific analysis showed that the relative isomer concentrations in adipose tissue at all time points were alpha>>gamma>beta which is in contrast to the test article composition (gamma>>alpha>beta). Steady state levels were achieved by study day 27. Levels in male and female rats were similar at all time points and declined during the recovery period.

All the test article-related changes at 100 and 300 mg/kg/day were mild, reversible, generally secondary to hepatic enzyme induction (which is an adaptive not a toxic change) and without effect on the clinical condition of the animals. The additional findings observed at 1000 mg/kg/day (increased gamma glutamyltransferase and additional increases in the size of the liver and prostate), were also reversible, not associated with specific target organ damage or diminished function and were, therefore, probably of limited, if any, toxicologic significance. On this basis the no-observed-adverse-effect level (NOAEL) of HBCD administered to Crl:CD®(SD)IGS BR rats by gavage in corn oil for 90 days is 1000 mg/kg/day *(Chengelis, C. An Oral (Gavage) 90 Day Toxicity Study of HBCD in Rats. Study No. WIL-186012. WIL Research Laboratories, Inc., Ashland, Ohio. 2001).*

4.3.2.3 Rat 28-Day Subchronic (BASF)

HBCD ("Hexabromid S") was tested in Sprague-Dawley rats (10/sex/group) at doses of 0, 1, 2.5 and 5% of the diet for 28 days. Doses calculated from the actual body weights and food consumption in this study are 0, 940, 2410, and 4820 mg/kg body weight/day.

No clinical signs related to treatment were observed at the 1% dose level. Body weights at the 1 and 2.5% dose levels were comparable to the controls. Liver weights (absolute and relative to body weight) were increased at all dose levels, but no microscopic pathology was detected. Thyroid hyperplasia was observed in some animals at all doses, and "very slight numerical development of the follicles and ripening follicles in the ovaries of females" at the high dose (4820 mg/kg/d) was reported. No changes in any other organ related to treatment and no changes in clinical chemistry tests were detected.

The report concluded that "The increased liver weight must be attributed to hyperactivity; hypermetabolism as a result of increased thyroid activity appears probable in view of the observations of the thyroid". Therefore, the increased liver weights were not pathologic: there were no microscopic lesions detected on histopathology and no change in clinical chemistry values *(Zeller H and Kirsch P (1969) Hexabromocyclododecane: 28-day feeding trials with rats. BASF Unpublished Laboratory Report)*.

Recent work on the relationship of liver weight, microsomal enzyme induction, and histological change in rat toxicology studies has been published (Amacher et al, Food and Chemical Toxicology, 36, 831-839, 1998). This paper concluded "The preponderance of data collected in these 11 studies indicates that microsomal enzyme induction was not accompanied by evidence of chemically-induced liver injury. We conclude that in the rat, both hepatomegaly and microsomal enzyme induction are benign and adaptive changes in response to certain chemicals that stimulate the hepatic drug metabolizing enzyme system."

4.3.2.4 Rat 90-Day Subchronic (BASF)

HBCD ("Hexabromid S") was tested in Sprague-Dawley rats at doses of 0, 0.16, 0.32, 0.64 and 1.28% of the diet for 90 days. Doses calculated on the actual body weights and food consumption in this study reveals: 0, 120, 240, 470 and 950 mg/kg body weight/day.

Doses up to 0.64% (470 mg/kg/d) produced no adverse clinical signs, no change in body weight, and no change in clinical chemistry results. An increase in the relative liver to body weight ratio was found, and was accompanied by fatty accumulation but no other histologically discernible changes were detected in the liver. Further, no histological changes were found in any other organ. The original report stated that in the "absence of detectable clinico-chemical disturbances or histological changes of the vital organs, it was concluded that the increased liver weight and the fat deposits, both of which were largely reversible when administration of Hexabromid S was stopped, were the result of a temporary increase in the activity of the liver." Thus, no adverse effect was produced at the highest dose tested, 1.28% of the diet *(Zeller H and Kirsch P (1970) Hexabromocyclododecane: 90-day feeding trials with rats. BASF Unpublished Laboratory Report).*

4.3.3 Genetic Toxicity – Mutation

HBCD did not induce genetic toxicity when tested in the Ames, *in vivo* mouse micronucleus, or *in vitro* chromosome aberration tests.

4.3.3.1 Ames Salmonella

HBCD has been tested for mutagenicity in the Ames Salmonella microsomal assay, both with and without metabolic activation, in multiple tests.. All results were negative *(Ogaswara S and Hanafusa T. (1993) Report on mutagenicity test on Pyroguard SR-103 using microorganisms; Baskin A and Phillips, B. (1977) Mutagenicity of two lots of FM-100, Lot 53 and residue of Lot 3322 in the absence and presence of metabolic activation. Industrial Biotest Laboratories, Sponsored by Velsicol Chemical Corporation; Anonymous. (1979) Mutagenicity test of GLS-S6-41A. Gulf South Research Institute, Sponsored by Ethyl Corporation; US Environmental Protection Agency (1990) Ames metabolic activation test to assess the potential mutagenic effect of Compound No. 49. Letter from BASF. EPA/OTS Doc #86-900000385; Simmons V., Poole, D., Newell, G., and Skinner, W. (1976) In vitro microbiological mutagenicity studies for four CIBA-GEIGY Corporation compounds. SRI Project LSC-5702.).*

4.3.3.2 *In Vivo* Mouse Micronucleus (BASF)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current OECD guidelines and Good Laboratory Practices.

HBCD dose levels administered intraperitoneally to male mice were 0, 500, 1,000 or 2,000 mg/kg body weight. The negative control animals were administered the vehicle, DMSO.

Cyclophosphamide and vincristine were used as positive controls and responded as expected. HBCD-treatment did not increase in number of polychromatic erythrocytes containing either small or large micronuclei. Micronuclei formation in HBCD-treated mice was within the same range as that of the concurrent negative control and within the range of historical control data. No evidence of chromosome damaging (clastogenic) effects was observed. There was no indication of any impairment of chromosome distribution in the course of mitosis. HBCD was clearly negative for clastogenicity and the ability to induce spindle poison effects in this mouse micronucleus test *(Engelhardt, G and Hoffmann, H. (2000) Laboratory Project Identification: 26M0100/004018. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen, Germany).*

4.3.3.3 In Vitro Iatrogenic Recombination

The Sp5 and SPD8 cell lines were developed by the paper's authors. The clones used in this study exhibit a spontaneous partial duplication of the hprt gene, resulting in a non-functional hgprt protein. These mutants revert spontaneously to a functional hprt gene phenotype by recombination with a frequency of 1 x 10^5 reversions/cell generation. This reversion frequency is said to increase by exposure to chemical or physical agents. Treatment with the test substance was for 24 hr at 37 degrees C. HBCD was tested *in vitro* in hamster cells (Sp5/V79 and SPD8) in a recombination assay at five doses between 2 and 20 ug/ml plus a control. In the SPD8 cells, HBCD concentrations of 0, 3, 6, 10, 15, and 20 ug/ml resulted in a reversion frequency of 1.0, 0.7, 0.8, 0.9, 1.4, and 1.9, respectively. Cytoxicity was observed at the 20 ug/ml dose. In the Sp5 cells, HBCD concentrations of 0, 2, 5, 10, 15, 20 ug/ml resulted in a reversion frequency of 1.0, 1.0, 0.8, 1.1, 1.4 and 2.2, respectively. Cytotoxicity was not observed. The reversion frequency at the 20 ug/ml dose for the Sp5 and SPD8 cells was statistically different from the control (Student's t test, $p<0.05$). Treatment with HBCD resulted in an ~ maximal 2-fold increase in revertant frequency. *(Helleday et al. Brominated flame retardants induce intragenic recombination in mammalian cells. Mutation Research 439 (1999) 137-147).*

This is a non-standard genetic toxicity test, and its reliability and predictive ability is unknown. This is not a test used by regulatory agencies to assess genotoxicity potential.

4.3.4 Genetic Toxcity – *In Vitro* Chromosome Aberration (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD and GLP guidelines.

HBCD was tested in the in vitro mammalian cytogenetic test using human peripheral blood lymphocytes both in the absence and presence of metabolic activation. The assay was performed in two phases. The first phase, the initial chromosome aberration assay, was conducted to establish the dose range for testing and to evaluate the clastogenic potential of the test article. The second phase, the independent repeat chromosome aberration assay, was performed to confirm the test system response to the test article seen in the initial assay.

Dimethylsulfoxide was used as a solvent. In the initial assay, the maximum dose tested was 2,500 ug/ml. Dose levels greater than 2,500 ug/ml were insoluble in treatment medium. Visible precipitate was observed in treatment medium at 750 and 2,500 ug/ml and was soluble but cloudy at dose levels of 75 and 250 ug/ml. The test article was soluble in treatment medium at all other doses tested. In the non-activated portion of the initial assay cells were exposed to the test article continuously for 20 hours; in the S9-activated portion of the initial chromosome aberration assay, cells were exposed to the test article for 4 hrs. Metaphase cells were collected at 20 hrs after initiation of treatment. Dose levels of 2,500 ug/ml in the non-activate study and 750 and 2,500 ug/ml in the S9-activated study were not analyzed for chromosome aberrations due to complete mitotic inhibition. Toxicity (mitotic inhibition) of ~56% was observed at the highest dose level (750 ug/ml) evaluated for chromosome aberrations, in the non-activated study. In the S9-

activated study, 13% toxicity was observed at the highest dose level (250 ug/ml) evaluated for chromosome aberrations. No statistically significant increases in chromosome aberrations were observed in either the non-activated or S9-activated test systems relative to the solvent control group regardless of dose level.

Based on the results of the initial assay, an independent repeat chromosome aberration assay was conducted in the absence and presence of an Arochlor-induced S9 metabolic activation system at dose levels of 10, 19, 38, 75, 150, 300 and 600 ug/ml. The test article was soluble but cloudy at 75 ug/ml and was workable in treatment medium at dose levels 150 ug/ml and higher. The test article was soluble in treatment medium at all other concentrations tested. In the independent repeat assay, cells were exposed to the test article continuously for 20 or 44 hr in the non-activated test system and for 4 hours in the S9-activated test system. Metaphase cells were collected for microscopic evaluation in both the non-activated and S9-activated studies at 20 and 44 hrs after initiation of treatment. Toxicity, measured by mitotic inhibition, was ~55% and 94% at the 20 and 44 hr harvests, respectively, at the highest dose levels (600 and 300 ug/ml) evaluated for chromosome aberrations in the nonactivated studies. In the S9-activated studies, toxicity was approximately 71% and 69% at the 20 and 44 hr harvests, respectively, at the highest dose levels (600 and 300 ug/ml) evaluated for chromosome aberrations. The 600 ug/ml dose level in the non-activated 44 hr harvest and in the S9-activated 20 hr harvest was not analyzed for chromosome aberrations due to an insufficient number of scorable metaphase cells. No statistically significant increases in structural chromosome aberrations were observed in either the non-activated or S9-activated studies, regardless of dose level or harvest time. No statistically significant increases in numerical chromosome aberrations were observed in either the non-activated or S9-activated studies at the 44 hr harvest time, regardless of dose level. HBCD was negative for the induction of structural and numerical chromosome aberrations in human peripheral blood lymphocytes *(Gudi, R. and Schadly, E. 1996. Laboratory Study Number G96AO61.342. Microbiological Associates, Inc., Rockville, MD).*

4.3.5 Developmental Toxicity Data

Two developmental toxicity studies at doses up to 1,000 mg/kg/d have been performed in the rat. Neither was positive for the induction of maternal or fetal toxicity or developmental effects.

4.3.5.1 Rat Prenatal Developmental Toxicity (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. The study was performed according to EPA, OECD and GLP guidelines. This study was required by KEMI (without consultation of the EU Technical Meeting) because KEMI decided the existing study in the literature (Murai et al.) was insufficient.

HBCD was administered in corn oil by gavage to 25 presumed pregnant Crl:CD(SD)IGS Br rats/group once daily from gestation days 6-19 at doses of 0, 250, 500 or 1,000

mg/kg/day. Control animals received corn oil only. Female rats were mated in-house and were treated daily on gestation days 6-19 with HBCD via gavage at dose levels of 0 (vehicle control), 250, 500 or 1000 mg/kg/day at a constant volume of 5 ml/kg. Individual doses were based on the most recent body weight. The day on which evidence of mating was observed was considered day 0 of gestation. Dams were observed daily and maternal body weight and food consumption measured at appropriate intervals. Females were euthanized on day 20 of gestation and necropsied. Gravid uterine and liver weights were recorded. Litters were delivered by cesarean section. The total number of corpora lutea, total number of implantations, early and late resorptions, number and location of all fetuses, and the sex and individual weights of fetuses were recorded. All fetuses were examined grossly. Approximately one-half of the fetuses in each litter were stained with Alizarin Red S and Alcian Blue and evaluated for skeletal/cartilaginous malformations and ossification variations. The maternal day 20 gestation examinations and cesarean sections, and subsequent fetal evaluations were performed blind to treatment.

No mortality occurred during the course of the study. No treatment-related clinical signs were observed. Body weight gain and food consumption were not adversely affected. No treatment-related findings were detected at necropsy. Intrauterine growth and survival were unaffected by treatment. No treatment-related fetal malformations or developmental variations were observed. The no-effect level (NOEL) for maternal toxicity and developmental toxicity was 1,000 mg/kg/day, the highest dose tested *(Stump, D. 1999. A Prenatal Developmental Toxicity Study of Hexabromocyclododecane (HBCD) in Rats. Laboratory Study No.: WIL-186009. WIL Research Laboratories, Inc., Ashland, OH.)*.

4.3.5.2 Rat Developmental Toxicity Study

Murai et al. 1985 (*Pharmacometrics (Japan) 29(6):981-986*) identified no reproductive or developmental effects in the rat at doses up to 1% in the diet administered from days 0-20 of gestation. This dose is approximately equivalent to 500 mg/kg/d.

The Murai et al study consisted of a 7 day dose range finding study (n=5 rats/dose group) and a combined teratogenicity-developmental study (n=20/dose group). Doses in the 7 day range finding study were 0, 0.3, 1, 3 or 10 g/kg/day. Doses as high as 10 g/kg/day produced no evidence of toxicity. A statistically significant ($P<0.01$) increase in liver weight was noted in groups receiving $\geq$ 1 g/kg/day. Doses for the combined teratogenicity-developmental study were based on this increase in liver weight. In the combined teratogenicity-developmental study, pregnant female rats were fed diets containing 0, 0.01, 0.1, or 1% HBCD on days 0-20 of gestation. Daily doses were estimated by the authors to be 0, 5, 50 or 500 mg/kg/day and the average total dose/rat/group was estimated to be 0, 0.13, 1.28 or 12.0 g/kg. Rats were observed daily and body weight and food consumption measured. Fourteen rats from each group were sacrificed on day 20 of gestation and their fetuses were examined for toxicity or teratogenicity. Approximately 150 fetuses/dose level were examined for evidence of teratogenicity. All fetuses from all litters were examined for signs of external anomalies.

Approximately 2/3 of the fetuses/dam were examined for skeletal abnormalities; the remaining fetuses from each dam were examined for any abnormalities of the internal organs. In addition, six rats from each group were allowed to deliver their litters and growth of the litters was observed until the 7th week post-parturition.

The authors' estimated the doses in the feed were equivalent to 0, 5, 50 or 500 mg HBCD /kg body weight /day. No adverse effects were detected in any treatment group with respect to maternal weight gain, food consumption, or gross appearance of internal organs. The mean liver (absolute and relative to body weight) weight in the 1% group was statistically different (higher) from the control mean. Normal development was seen in neonates carried through to six weeks of age.

There was no adverse effect of treatment on the number of corpora lutea, implants, resporptions, live fetuses, sex ratio, or body or placental weight. No fetal deaths occurred in any group. No external, skeletal or visceral malformations were detected. A few skeletal variations were detected but where of similar types and numbers in the control and treated groups.

There was no significant differences between the control and treated groups in the number of implantation, live newborns, dead newborns, live newborn parturition index. The weaning and survival index was comparable in the control and treated groups. Body weight changes in the newborns was comparable in all groups.

No reproductive or developmental effects where detected in rats at HBCD doses up to 1% in the diet (~500 mg/kg/d) administered from days 0-20 of gestation. Further, normal development was seen in neonates carried through to six weeks of age.

Dose levels: 0, 0.01, 0.1, or 1% HBCD on days 0-20 of gestation [Murai estimate: 0, 5, 50 or 500 mg/kg/day]. No teratogenic effects. Normal development in neonates carried through age 6 wks. NOEL = 1% of diet *(Murai, T. Kawasaki, H., Kanoh, S. 1985. Studies on the toxicity of insecticides and food additives in pregnant rats - fetal toxicity of Hexabromocyclododecane. Pharmacometrics (Japan) 29(6):981-986).*

4.3.6 Reproductive Toxicity Data

Two teratology studies on HBCD are available; one published in the literature (high dose = 1% of the diet) and one recently completed by industry under current guidelines and Good Laboratory Practices using the HBCD in commercial production and use (high dose = 1000 mg/kg/d). Both studies are negative for developmental toxicity. Repeated dose studies (two 28 day studies, one 90 day study, and one 18 month study in a second species) indicate HBCD does not affect the reproductive organs at doses up to 1000 mg/kg/day. According to the SIDS Manual, when teratology and 90 day studies show no effects on the reproductive system then the requirement for the reproductive endpoint are met. Teratology, 28 day, 90 day and 18 month studies all demonstrate HBCD has no effect on the reproductive system at the limit dose of 1000 mg/kg/d.

4.3.7 Additional Toxicology Data

4.3.7.1 Pharmacokinetics

There are least two pharmacokinetic studies were performed in Japan in the early 1980s, as well as one from Velsicol (1980). One Japanese study used gas chromatography for the analyses and therefore the results are questionable (R. Arita et al. 1983). The other Japanese study reportedly used ^{14}C-labelled material and may be of more value. The Velsicol study reported that HBCD was absorbed and metabolized extensively with ~86% eliminated in 72 hrs.

The 2001 90 day study sponsored by BFRIP showed very different levels of the three stereoisomers from that administered in the test article.

Based on this limited data, HBCD would appear to be well absorbed and metabolized prior to elimination, but it is unclear how and to what extent. The three stereoisomers are likely handled differently in the mammalian system.

4.3.7.2 Carcinogenicity: 18-Month Mouse Carcinogenicity

Male and female mice were fed diets containing HBCD at 0, 100, 1000 or 10,000 ppm for 18 months. There was no evidence of carcinogenicity at any dose level. This study was performed by the Department of Toxicology, National Public Health Research Institute, Biological Safety Test and Research Center, Japan (date not specified).

4.3.7.3 Skin Sensitization

Four sensitization studies have been conducted; three in guinea pigs and one in human volunteers. The 1997 guinea pig maximization test performed by BFRIP was negative. The Momma et al. *(Pharmacometrics, 1985, 29:981-986)* and Nakamura et al. *(Contact Dermatitis, 1994, 31:72-85)* studies reported in the literature were positive; the test article appears to have been an HBCD product produced in Japan. The patch test in human volunteers was negative.

4.3.7.3.1 1972 Human Patch Test (DuPont)

The test samples were Tyvek T-12 with 10% HBCD. One inch squares of the test samples were applied to the arms of 10 men and to the arms or legs of ten women and held in place with Dermicel tape for six days. After a two-week rest period, new patches were applied for 48 hours as a challenge test for skin sensitization. Skin under the patches was examined at two and six days after the first application and on removal of the challenge patch. No skin reactions were observed on any subject at any examination *(McDonnell, M. 1972. Haskell Laboratory Report No. 185-72. Haskell Laboratory for Toxicology and Industrial Medicine).*

4.3.7.3.2 Guinea Pig Skin Sensitization Tests

The 1997 Guinea Pig Maximization Skin Sensitization Test performed by BFRIP used a test article which was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. The study was conducted according to EPA, OECD and GLP guidelines. The test article used in this study was representative of the HBCD commercial product sold in the U.S. The test was negative for the induction of skin sensitization *(Wenk, M. 1996. Maximization Test in Guinea Pigs. Test Article: Hexabromocyclododecane. Project No. M96AO61.1X64. Microbiological Associates, Inc. Rockville, MD).*

The Momma (1985) and Nakamura (1994) studies, which produced positive results, used an HBCD product manufactured in Japan.

The reason for the discrepancy between these results is not apparent. However, the negative results in the 1997 test that used the highest possible concentration for topical induction and challenge, raise questions about the potential for HBCD to produce even a mild sensitization reaction in humans. The methodologies used in these 3 sensitization tests are provided in Table 2.

TABLE 2. Comparison of the methodology used in 3 guinea pig skin sensitization studies conducted on HBCD.

	BFRIP, 1997	MOMMA, 1985	NAKAMURA, 1994
INDUCTION – ID			
VOLUME	0.1 ml	0.05 ml	Assume 0.05 ml ?
CONCENTRATION	5%	0.05, 0.5, 5%	0.5, 5%
DOSE	0.005 mg	0.000025, 0.00025, 0.0025 mg	0.00025, 0.0025 mg
VEHICLE	Corn oil	Olive oil	Olive oil
INDUCTION – TOPICAL			
AMOUNT	500 mg	200 mg	Assume 200 mg ?
CONCENTRATION	100%	25%	25%
DOSE	250 mg	50 mg	50 mg
VEHICLE	Corn oil*	Vaseline	Petrolatum
CHALLENGE			
VOLUME/AMOUNT	500 mg	0.02 ml	0.1 ml
CONCENTRATION	100%	0.005, 0.05, 5%	0.05, 0.5, 5%
DOSE	250 mg	0.000001, 0.00001, 0.0001, 0.001 mg	0.00005, 0.0005, 0.005 mg
VEHICLE	Corn oil*	Acetone	Acetone

* Only moistened with corn oil.

5.0 HBCD TESTING PLAN

A complete set of SIDS-level data currently exists for HBCD (Table 3), and the results are described in the attached robust summaries. Therefore, no testing is planned under this program.

TABLE 3. HBCD Test Plan Summary.

Study Type	Data Available	Data Acceptable	Estimation	Testing Required
Physical/Chemical				
Melting Point	Y	Y	-	N
Boiling Point	N	-	-	N
Vapor Pressure	Y	Y	-	N
Water Solubility	Y	Y	-	N
Environmental Fate				
Photodegradation	N	-	Y	N
Stability in Water	N	-	Y	N
Biodegradation	Y	Y	-	N
Transport (Fugacity)	N	-	Y	N
Ecotoxicity				
Acute Toxicity to Fish	Y	Y	-	N
Acute Toxicity to Aquatic Invertebrates	Y	Y	-	N
Toxicity to Aquatic Plants	Y	Y	-	N
Toxicology Data				
Acute Toxicity	Y	Y	-	N
Repeated Dose Toxicity	Y	Y	-	N
Genetic Toxicity – Mutation	Y	Y	-	N
Genetic Toxicity – Chromosome Aberration	Y	Y	-	N
Developmental Toxicity	Y	Y	-	N
Reproductive Toxicity	Y	Y	-	N

Sponsor	[redacted]	Albemarle Corporation	Create Date: [redacted]
CAS Number	[redacted]	Cyclododecane, 1,2,5,6,9,10-hexabromo-	
Consortium	[redacted]	CMA Brominated Flame Retardant Industry Panel (BFRIP)	

Select an End Point: TO - Genetic toxicity in vivo

[redacted]

Physical/Chemical Properties

- ✔ Melting Point
- Boiling Point
- ✔ Partition Coefficient
- ✔ Vapor Pressure
- ✔ Water Solubility

Environmental Fate

- Photodegradation
- Stability in Water
- ✔ Biodegradation
- ✔ Transport

Ecotoxicity

- ✔ Acute Toxicity to Fish
- ✔ Toxicity to Aquatic Plant
- ✔ Acute Toxocity to Aquatic Invertebrates

Health

- ✔ Acute Toxicity
- ✔ Genetic Toxicity in Vivo
- ✔ Repeat Dose Toxicity
- ✔ Genetic Toxicity in Vitro
- Reproductive Toxicity
- ✔ Developmental Tox/Teratogenicity

[redacted]

EPA High Production Volume (HPV) Track

Physical-Chemical End Point:
Melting Point

Sponsor ID	119002	Albemarle Corporation	Create Date	4/6/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y

Revision Date:
12/5/01

Test Substance

Remarks The test substance consisted of various commercial products.

Chemical Category

Method

>> Method/Guideline followed

Not specified.

>> GLP Unknown

>> Year study performed 1994

Remarks for Method

Results

>> Precision range

>> Melting Point Value 175

>> Upper Value 195

>> Unit °C

EPA High Production Volume (HPV) Track

Physical-Chemical End Point: Melting Point

Sponsor ID	[illegible]	Albemarle Corporation		Create Date	[illegible]
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-		Study Number	[illegible]
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)		Completed:	Y

>> Decomposition Yes

>> Sublimation No

Results Remark Various melting points have been reported for different products: 175-183 degrees C (Saytex HBCD-LM); 187-195 degrees C (Saytex-HM), 190 degrees C (GLCC product).

Conclusions

HBCD is a solid at room temperature whose melting point varies with composition.

Data Quality

Reliability Good

Data Reliability Remarks

The melting point data was provided by commercial manufacturers of the substance.

Reference

>> Remarks IUCLID Dataset. Substance ID: 25637-99-4. 18-Feb-2000.

EPA High Production Volume (HPV) Track

Physical-Chemical End Point: Melting Point

Sponsor ID	1100021	Albemarle Corporation	Create Date	12/03	
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	3	
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y	

General

EPA High Production Volume (HPV) Track

Physical-Chemical End Point:
Partition Coefficient

Sponsor ID	1100021	Albemarle Corporation	Create Date	11/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y

Revision Date:
12/5/01

Test Substance

Remarks

The test article was a composite of equal parts of the commercial hexabromocyclododecane (HBCD) commercial product produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. The test article composite was analyzed for characterization and homogeneity. The results of the analysis indicated the test article was homogeneous and contained the following components: HBCD beta isomer 8.5%, HBCD alpha isomer 6.0%, HBCD gamma isomer 79.1%.

Chemical Category

Method

>> Method/Guideline followed

OPPTS 830.7560 Partition Coefficient (n-Octanol/Water), Generator Column Method

>> GLP Yes

>> Year study performed 1997

Remarks for Method

A single generator column was prepared for the definitive test. The column was packed with Chromosorb W HP support and loaded with an approximate 0.2% solution of the test substance in octanol. Dilutions of the test substance solution in octanol were analyzed. The column termperature was maintained at 25 +/- 0.05 degrees C and reagent water saturated with octanol was pumped through it at approximately 1 mL per min to elute the test substance. Samples of the eluate were collected and analyzed to determine the concentration of the test substance in the aqueous fractions.

The analytical method consisted of extracting the aqueous samples with dicloromethane (DCM), evaporating the DCM, and reconstituting the sample residues in acetonitrile/water (50:50, v/v).

Results

>> Precision =

>> Value of Log Pow 5.625

EPA High Production Volume (HPV) Track

Physical-Chemical End Point:
Partition Coefficient

Sponsor ID	1100021	Albemarle Corporation	Create Date	1/6/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed	Y

>> Upper Value 0

>> Temperature 25 degrees C

Results Remark

HBCD's water solubility was previously determined to be 0.0034 mg/L (Stenzel and Markley, 1997).

No interferences were observed at or above the limit of quantitation in the matrix blank sample. The percent recovery of the 1.00 and 10.0 ug HBCD/L matrix fortifications were 104 and 85%. The mean recovery was calculated at 95% of nominal.

The nominal flow rate of reagent water through the generator column was measured prior to the start of sample collection. Flow rates were also calculated based on the volume and collection time of each sample that was analyzed. The pump setting was 1.0 mL/min and the flow rate was measured at 1.0 mL/min. The calculated flow rates for samples averaged 0.87 mL/min and ranged from 0.86 to 0.87 mL/min.

The mean concentration of HBCD measured in the aqueous samples eluted from the generator column was 3.97 ug HBCD/L or 6.19 x 10-9 M (molecular weight of HBCD is 641.7 g/mole).

The mean concentration of HBCD measured in the octanol stock solution samples was 1.67 g HBCD/L or 2.61 x 10-3 M (molecular weight of HBCD is 641.7 g/mole).

Conclusions

The octanol/water partition (Kow) coefficient was calculated from the following equation:

$$Kow = \frac{\text{Measured Concentration in Octanol (M)}}{\text{Measured Concentration in Aqueous Samples (M)}}$$

Based on the results from octanol samples collected from the stock solution and aqueous samples collected from the generator column, the mean octanol/water partition coefficient (Kow) for HBCD was determined to be 4.22 x 10-5 (log Kow = 5.625).

Data Quality

Reliability High

Data Reliability Remarks

EPA High Production Volume (HPV) Track

Physical-Chemical End Point: Partition Coefficient

Sponsor ID	1100021	Albemarle Corporation	Create Date	10/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y

This study was performed according to current guidelines and Good Laboratory Practices by a laboratory with considerable experience with these studies. Extensive attention was paid to analytical method development and performance.

Reference

>> Remarks

MacGregor, J and Nixon, W. (1997) Hexabromocyclododecane (HBCD): Determination of n-Octanol/Water Partition Coefficient. Project Number: 439C-104. Wildlife International LTD, Easton, MD.

General

Study sponsored by the Chemical Manufacturers Association Brominated Flame Retardant Industry Panel, Arlington, VA.

EPA High Production Volume (HPV) Track

Physical-Chemical End Point:
Vapor Pressure

Sponsor ID	110002	Albemarle Corporation	Create Date	5/7/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	110012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed	Y

Revision Date: 12/5/01

Test Substance

Remarks The test article was a composite of equal parts of the commercial hexabromocyclododecane (HBCD) commercial product produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. The test article composite was analyzed for characterization and homogeneity. The results of the analysis indicated the test article was homogeneous and contained the following components: HBCD beta isomer 8.5%, HBCD alpha isomer 6.0%, HBCD gamma isomer 79.1%.

Chemical Category

Method

>> Method/Guideline followed

OECD Method 104; U.S. EPA OPPTS 830.7950 Vapor Pressure

>> GLP Yes

>> Year study performed 1997

Remarks for Method

The objective of this study was to determine the vapor pressure of HBCD at ambient temperature using a spinning rotor gauge (SRG). The SRG method was chosen due to the extremely low vapor pressure anticipated for this substance.

The SRG system was configured with an empty 10 mL beaker in the sample chamber to make control measurements. The system baseline pressure and out-gassing rate were each measured twice.

A sample of the test substance in a 10 mL beaker was placed in the sample chamber. The SRG system was used to monitor the steady-state pressure of the sample while the system was open to the vaccum pumps, and the pressure increase from the sample while the valve was closed to the pumps. The steady-stae pressure and pressure increase measurements were repeated three times.

Results

>> Precision =

>> Vapor Pressure Value 0.0000627

EPA High Production Volume (HPV) Track

Physical-Chemical End Point: Vapor Pressure

Sponsor ID	1100021	Albemarle Corporation	Create Date	1/6/01
CAS Number	3194-55-6	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	1101017	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed	Y

>> Upper Value 0

>> Unit Pascals

>> Temperature 21 degrees C

>> Decomposition No

Results Remark

The baseline pressure of the system containing an empty beaker was determined to be less than 1 x 10E-7 Pa for both measurements. The technical specifications of the SRG indicated the low end of the measurement range to be 1 x 10E-5 Pa. The baseline pressure was considered to be essentially zero, and indicated the system was free of contaminaiton. The out-gassing rate (slope) was <1 x 10E-7 Pa/sec for both measurements. The out-gassing rate indicated there were no leaks in the system.

The mean steady-state pressue of the HBCD sample was 6.166 x 10E-5 based on three separate determinations. The slope of the line fit to the pressue increase data was less than the out-gassing rate of the empty system for each determination, indicating the system had achieved saturation of the gas from the HBCD sample and was leak-free. The intercept was only slightly greater than the steady-state pressure. The temperature of the system averaged 21 degrees C.

The vapor pressure for each determination of the HBCD sample was calculated from the following equation:

Vapor Pressure = intercept of sample - mean intercept of empty system.

The mean vapor pressure of HBCD was determined to be 6.27 x 10E-5 Pa with a standard deviation of 0.21 x 10E-5.

The vapor pressures of di(2-ethyl-hexyl)phthalate and hexachlorobenzene were measured using the same SRG system and determined to be 4.3 x 10E-5 Pa and 1.6 x 10E-3 Pa, respectively. Both of these measurements were consistent with ranges found in the literature.

Conclusions

EPA High Production Volume (HPV) Track

Physical-Chemical End Point: Vapor Pressure

Sponsor ID	1100022	Albemarle Corporation	Create Date	3/6/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y

Based on the results from three sets of measurements collected from the spinning rotor gauge, the vapor pressure of HBCD was determined to be 6.27 x 10E-5 Pa at 21 degrees C.

Data Quality

Reliability High

Data Reliability Remarks

This study was performed according to current guidelines and Good Laboratory Practices by a laboratory with considerable experience with these studies. Extensive attention was paid to analytical method development and performance.

Reference

>> Remarks

Stenzel, J and Nixon, W. (1997) Hexabromocyclododecane (HBCD): Determination of the Vapor Pressure Using a Spinning Rotor Guage. Project Number: 439C-117. Wildlife International LTD, Easton, MD.

General

Study sponsored by the Chemical Manufacturers Association Brominated Flame Retardant Industry Panel.

EPA High Production Volume (HPV) Track

Physical-Chemical End Point: Water Solubility

Sponsor ID	1100021	Albemarle Corporation	Create Date	1/e01	
CAS Number	3194-55-6	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1	
Consortia ID	110012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed	N	

Revision Date: 12/5/01

Test Substance

Remarks: The test article was a composite of equal parts of the commercial hexabromocyclododecane (HBCD) commercial product produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. The test article composite was analyzed for characterization and homogeneity. The results of the analysis indicated the test article was homogeneous and contained the following components: HBCD beta isomer 8.5%, HBCD alpha isomer 6.0%, HBCD gamma isomer 79.1%.

Chemical Category

Method

>> Method/Guideline followed

OECD Method 105, U.S. EPA 40 CFR Ch. 1 Section 796.1860 Water Solubility- Generator Column Method

>> GLP: Yes

>> Year study performed: 1997

Remarks for Method

This study was performed according to OECD Method 105 and U.S. EPA 40 CFR Ch. 1 Section 796.1860 Water Solubility- Generator Column Method.

A generator column was prepared. The column temperature was maintained at 25.0 degrees C and reagent water was pumped through it at approximately 2 mL per minute to elute the test substance. Samples of the eluate were collected and analyzed to determine the saturation concentration of the test substance. The flow rate of reagent water through the column was reduced to approximately half the original flow rate and the saturation concentration determined again.

Results

>> Precision: <

>> Water Solubility Value: 1

>> Upper Value: 0

EPA High Production Volume (HPV) Track

Physical-Chemical End Point: Water Solubility

Sponsor ID	160021	Albemarle Corporation	Create Date	1/x/01	
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number		
Consortia ID	101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	N	

>> Unit mg/L

>> Temperature 25 degrees C

>> Solubility Category Insoluble

>> pH Value 8 **>> pKa Value** 0

Results Remark

No interferences were observed at or above the limit of detection (0.5 ug HBCD/L) in any of the matrix blank or reagent blank samples. The peak area response for the matrix blanks was always below the response of the lowest calibration standard. The mean recovery from 10 matrix samples fortified at 10 ug/L was 105% (standard deviation 2.0), and ranged from 103% to 108%. The mean recovery from 10 matrix samples fortified at 1 ug/L was 104% (standard deviation 5.2),a nd ranged from 100% to 110%. The 1 ug/L concentration was considered the limit of quantiation.

A brief description of the analytical method is as follows: samples were extracted using dichloromethane (DCM). The DCM was evaporated to dryness and 1.0 ml o facetonitrile/water (50:50) was added. The samples was analyzed using HPLC/UV.
The nominal flow rate of reagent water through the generator column was initially set at 1.0 mL/min. The initial flow rate was measured at 2.0 mL/min prior to the start of sample collection. Samples were collected at this flow rate until the solubility plateau was achieved. The calculated flow rates for samples collected at the initial flow rate average 1.96 mL/min (range 1.88-1.98 mL/min). After the solubility plateau was achieved, the flow rate was reduced to ~half the initial flow rate. The reduced flow rate was measured at 1.0 mL/min prior to resuming sample collection. The calculated flow rates averaged 0.92 mL/min (range 0.91-0.93)

All samples collected at a nominal flow rate of 2.0 mL/min were analyzed and the solubility limit was considerated to have been achieved when at least 5 consecutive samples gave similar results. The mean concentration in samples meeting this criteria was 0.0034 mg/L with a standard deviation of 0.23.

The results from analyses of samples eluted at a nominal flow rate of 1.0 mL/min found a mean

Sponsor ID	1100021	Albemarle Corporation	Create Date	1/6/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	N

HBCD concentration of 0.0033 mg/L with a standard deviation of 0.20.

The pH of the water obtained from Wildlife International Ltd's well in February/March 1997 had a mean pH of 8.3 (range 8.2-8.4).

NOTE: HBCD has no ionizable groups and therefore the pKa value does not apply. A value of 0 was entered in the "pka value" field becuase this was a mandatory numeric field.

Conclusions

The solubility of HBCD in water was determined to be 0.0034 +/- 0.2 mg/L at 25 degrees C.

Data Quality

Reliability High

Data Reliability Remarks

This study was performed according to current guidelines and Good Laboratory Practices by a laboratory with considerable experience with these studies. Extensive attention was paid to analytical method development and performance.

Reference

>> Remarks

Stenzel, J. And Markley, B. (1997) Hexabromocyclododecane (HBCD): Determination of the Water Solubility. Project Number: 439C-105. Wildlife International LTD, Easton, MD.

General

Study sponsored by the Chemical Manufacturer's Association Brominated Flame Retardant Industry Panel, Arlington, VA.

EPA High Production Volume (HPV) Track

Environmental Fate and Pathway End Point: Biodegradation

Sponsor ID	[illegible]	Albemarle Corporation	Create Date	[illegible]
CAS Number	[illegible]	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y

Revision Date: 12/5/01

Test Substance

Remarks: The test article was a composite of equal parts of the commercial hexabromocyclododecane (HBCD) commercial product produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. The test article composite was analyzed for characterization and homogeneity. The results of the analysis indicated the test article was homogeneous and contained the following components: HBCD beta isomer 8.5%, HBCD alpha isomer 6.0%, HBCD gamma isomer 79.1%.

Chemical Category

Method

>> Method/Guideline followed

EPA OPPTS Method 835.3200: Ready Biodegradability, Closed Bottle Test; OECD Guideline 301D

>> Test Type

aerobic

>> GLP: Yes

>> Year study performed: 1996

>> Contact Time: 28

>> Inoculum

activated sludge, domestic, adapted

Remarks for Method

The test contained an inoculum control group, a reference group and a treatment group. The blank control, reference, and treatment groups contained ten replicate test chambers. The inoculum control was used to measure the dissolved oxygen consumption of the inoculum and was not dosed with a carbon source. The reference chambers were dosed with sodium benzoate, a substance known to be biodegradable, at a concentration of 2 mg/L. The treament group test chambers were used to evaluate the test substance at 7.7 mg/L. Measurements of oxygen consumption were performed on two test chambers from the control, reference and treatment groups on days 0, 7, 14, 21, and 28.

The test inoculum was secondary clarifier supernatant collected from Prospect Bay Wastewater Treatment Facility, Grasonville, MD. The theoretical oxygen demand value used to calculate the percent degradation of the test substance was 0.75 mg O2/mg.

Results

EPA High Production Volume (HPV) Track

Environmental Fate and Pathway End Point: Biodegradation

Sponsor ID	[illegible]	Albemarle Corporation	Create Date	1/6/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	110012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y

>> Precision =

>> Degradation Value 0

>> Upper value 0

>> Time Frame 28

>> Time Units Days

>> Breakdown products No

Results Remarks

The temperature range recorded during the test was 18-20 degrees C. The result of the standard plate count performed on the inoculum was 3.7 x 10E4 CFU/ml.

The average oxygen uptake exhibited by the control, reference, and treatment groups was measured at 0, 7, 14, 21 and 28 days. The oxygen depletion of the inoculum control was less than or equal to 1.5 mg O2/L. Degradation of the test substance was not observed over the 28-day test period.

The viability of the inoculum and validity of the test was supported by the results of the reference substance, sodium benzoate, degrading approximately 94%. An average percent biodegradation of > 60% was achieved by day 7, thereby fulfilling the criteria for a valid test.

Conclusions

Degradation of the test substance, HBCD, at 7.7 mg/L was not observed over the 28-day test period.

EPA High Production Volume (HPV) Track

Environmental Fate and Pathway End Point: Biodegradation

Sponsor ID	110021	Albemarle Corporation	Create Date	12-01
CAS Number	3194-55-6	Cyclododecane 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	110012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y

Data Quality

Reliability High

Data Reliability Remarks

This study was performed according to current guidelines and Good Laboratory Practices by a laboratory with considerable experience with these studies. Extensive attention was paid to analytical method development and performance.

Reference

>> Remarks

Schaefer, E and Haberlein, D. (1996) Hexabromocyclododecane (HBCD): Closed Bottle Test. Project No.: 439E-102. Wildlife International Ltd. Easton, MD.

General

Study sponsored by the Chemical Manufacturers Association Brominated Flame Retardant Industry Panel.

EPA High Production Volume (HPV) Track

Environmental Fate and Pathway End Point: Transport between Environmental Compartments (Fugacity)

Sponsor ID	[illegible]	Albemarle Corporation	Create Date	[illegible]	
CAS Number	[illegible]	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1	
Consortia ID	[illegible]	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y	

Revision Date: 12/18/01

Test Substance

Remarks: Hexabromocyclododecane (HBCD)

Chemical Category

Method

>> Method/Guideline followed

Developed by D. Mackay and co-workers

>> Test Type: Level III fugacity model

>> Year study performed: 2001

Remarks for Method

Model Used: Level III Fugacity Model (Full-Output), EPIWIN V3.04

Input parameters: chemical structure only; model default parameters accepted; model basd on emissions of 1000 kg/hr each to air, water and soil.

Results

>> Media

Air: 0.000685%; Water: 2.06%; Soil: 40.1%; Sediment: 57.9%

>> Distribution Concentration

Not provided by model.

EPA High Production Volume (HPV) Track

Environmental Fate and Pathway End Point: Transport between Environmental Compartments (Fugacity)

Sponsor ID	100021	Albemarle Corporation	Create Date	4/6/01
CAS Number	3194-55-6	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y

Results Remark

Estimated by model:
Soil Koc: 2.25 x 10+7
Vapor Pressure: 1.68 x 10-8 mmHg
Liquid VP: 5.74 x 10-7 mm Hg (super-cooled)
Melting Pt: 180 deg C
Log Kow: 7.74
Henry's LC: 6.43 x 10-11 atm-m3/mole

Conclusions

If released at equal rates to air, water and soil, HBCD is predicted to partition primarily to sediment (appr. 58%) and soil (appr. 40%). Only appr. 2% would partition to water with only trace (appr. 0.0007%) amounts to air. Appr. 90% would be reacted with only appr. 11% advected.

The model was also run 7 times using all permutations of air, water and soil emission rates as either 0 or 1000 kg/hr. The results were as follows. If released solely to air, the model predicted HBCD would partition appr. two-thirds to soil and one-third to sediment; 97% reacted. If released solely to water, HBCD would partition to sediment; total reacted = 71%. If released solely to soil, HBCD would remain in soil; total reacted = 100%. If released at equal rates to both air and water, HBCD would partition two-thirds to sediment and one-third to soil; total reacted = 84%. If released at equal rates to both air and soil, HBCD would partition appr. 80% to soil and 12% to sediment; total reacted = 98%. If released to water and soil, HBCD would partition two-thirds to sediment and one-third to soil; total reacted = 86%.

Based on the above, HBCD is not expected to move from water, soil or sediment to air. Furthermore, HBCD is not expected to move from soil into water.

Data Quality

Reliability High

Data Reliability Remarks

Reference

EPA High Production Volume (HPV) Track

Environmental Fate and Pathway End Point: Transport between Environmental Compartments (Fugacity)

Sponsor ID	100021	Albemarle Corporation	Create Date	1/6/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	100012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y

>> Remarks

Level III Fugacity Model, EPIWIN V3.04, Syracuse Research Corporation, Syracuse, NY.

General

EPA High Production Volume (HPV) Track

Ecotoxicity End Point:
Acute Toxicity to Fish

Sponsor ID	[illegible]	Albemarle Corporation	Create Date	[illegible]6.01	
CAS Number	[illegible]56	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1	
Consortia ID	110112	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed	N	

Revision Date
12/5/01

Test Substance

Remarks
The test article was a composite of equal parts of the commercial hexabromocyclododecane (HBCD) commercial product produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. The test article composite was analyzed for characterization and homogeneity. The results of the analysis indicated the test article was homogeneous and contained the following components: HBCD beta isomer 8.5%, HBCD alpha isomer 6.0%, HBCD gamma isomer 79.1%.

Chemical Category

Method

>> Method/Guideline followed
OECD Method 203

>> Test Type
flow-through

>> GLP Yes

>> Year study performed 1997

>> Species
Oncorhynchus mykiss

>> Analytical monitoring HPLC/UV/VIS Detector; LOQ=0.04 ug/l

>> Exposure period 96 hours

>> Statistical Method None needed - no mortality observed.

Remarks for Method
This study was performed according to OECD Method 203 and TSCA Title 40 of CFR, Part 797, Section 1400. Rainbow trout were exposed to one of five test concentrations, a solvent control, or the negative (well water) control. Two replicate test chambers were maintained in each treatment and control group. Ten rainbow trout were used in each test chamber for a total of 20 rainbow trout per test concentration. Nominal test concentrations were selected in consultation with the Sponsor, and were based on the solubility of the test compound in water (3.4 ug/L) and the results of an expoloratory rangefinding test. Due to co-eluting artifacts at 96

hrs, mean measured test concentrations were determined analytically from samples of test water collected from each treatment and control group at the beginning of the test and at approximately 48 hrs.

The selection of exposure concentrations took into consideration the water solubility limit and a finding of no acute toxicity from an exploratory rangefinding test. The water solubility limit was determined in a generator column elution study to be 3.4 ug/L. However, there was a potential to have a slight enhancement of HBCD's water solubility due to the use of dimethylformamide (DMF) as a vehicle in the diluter system. For this reason, the highest test concentration selected for the acute toxicity test was twice the defined solubility limit (i.e., 6.8 ug/L). The series of 5 nominal test concentrations used in the test were 1.5, 2.2, 3.2, 4.6 and 6.8 ug/L. In this way, the solubility limit of HBCD was bracketed by the five concentrations.

Delivery of the test substance was initiated approximately 6 days prior to the introduciton of the fish to the test water in order to achieve equilibrium of the test substance in the test chambers. The fish wree indiscriminately assigned to exposure chambers at test initiation. Observations of mortality and other clinical signs were made approximately 1, 24, 48, 72 and 96 hrs after test initiation. The no mortality concentration and no observed effect concentration (NOEC) were determined by visual interpretation of the mortality and clinical observation data.

All fish were from the same source and year class, and the total length of the longest fish was no more than twice the length of the shortest. The average length of 10 negative control fish at the end of the test was 55 mm with a range of 50-61 mm. The wet weight of 10 negative control fish at the end of the test was 2.4 g with a range of 1.6-3.6 g. Loading, defined as the total wet weight of ifsh per liter of test water that passed through the test chamber in 24 hrs, was 0.27 g fish/L/day.

Temperature, dissolved oxygen, and pH were measured. Temperatues were within the limits of the 12 +/- 2 degrees C range established for the test. Dissolved oxygen concentrations were greater than or = 78% of saturation throughout the test. Water pH ranged from 8.2-8.3. Total organic carbon values were <1.0 mg C/L at test initiation and termination.

Test substance concentrations were determined via HPLC using a UV/VIS detector.

Results

>> Nominal concentration	0, 0.0015, 0.0022, 0.0032, 0.0046, 0.0068
>> Measured concentration	0, 0.00075, 0.0015, 0.0023, 0.0023, 0.0025
>> Precision	>
>> Endpoint Type	LC0

EPA High Production Volume (HPV) Track

Ecotoxicity End Point:
Acute Toxicity to Fish

Sponsor ID	[illegible]	Albemarle Corporation	Create Date	[illegible]	
CAS Number	[illegible]	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1	
Consortia ID	[illegible]	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	N	

>> Endpoint Value 0 **>> Unit used** mg/L

>> Concentration Type Nominal **>> Endpoint Time** 96

>> Statistical results

None needed - no mortality observed.

Results Remark

One set of pretest water samples was collected from the highest and lowest test concentrations and analyzed for HBCD concentrations. All pretest samples yielded concentrations that were considerably lower than the expected concentrations. The toxicity test was initiated and measurements of the HBCD concentrations in all test chambers were made at the beginning, middle and end of the test. In general, concentrations of HBCD made on samples collected at Day 0 and Day 2 were variable and failed to correspond to the dilution series expected from the nominal concentrations. All diluter operational records were checked and no evidence of any malfunctions or errors were found. Concentrations measured in the Day 4 samples were artificially high due to co-eluting artifacts at the retention time of HBCD. Attempts were made to separate the co-eluting artifacts during a reanalysis of the orginal Day 4 sample extracts, but the resulting chromatography showed those same interferences.

While the pattern of measured HBCD was unexpected, the results suggest that the exposure solutions were at the solubility limit of HBCD in the diluter system. The variability in the measured concentrations could have been influenced by the temperature of the exposure water (12 degrees C), the flow-through design, or the hydrophobic nature of HBCD (as evidenced by its nonpolar alkane structure and extremely low water solubility). These factors could explain both the failure of the measured values to correspond to the nominal concentrations and the variability observed in the measured concentrations. Overall, it appears that the solubility limit of HBCD, under the conditions that it was applied in this test, is within the range of 2.0 - 3.0 ug/L. The values obtained in the Day 4 samples were not reflective of the true conditions due to the co-eluting artifacts, and therefore, were not used in the study.

Temperatures were within the limits of the 12 +/- 2 degrees C range established for the test. Dissolved oxygen concentration of > or = 78% of saturation were observed throughout the test. Water pH was consistent with values for moderately-hard water and ranged from 8.2 to 8.3. Total organic carbon values were < 1.0 mg C/L at test initiation and termination.

Observations for mortality and other signs of toxicity were made daily. Rainbow trout in the negative control and solvent control groups appeared healthy and normal throughout the test. All rainbow trout in the 1.5, 2.2, 3.2, 4.6 and 6.8 ug/L (nominal) treatment groups also appeared normal throughout the test with no mortalitites or overt signs of toxicity. Based on these results, the LC50 values at 24, 48, 72 and 96 hours were estimated to be >6.8 ug/L, the highest concentration tested.

EPA High Production Volume (HPV) Track

Ecotoxicity End Point:
Acute Toxicity to Fish

Sponsor ID	[illegible]	Albemarle Corporation	Create Date	[illegible]	
CAS Number	[illegible]	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1	
Consortia ID	[illegible]	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed	N	

Conclusions

The 96-hour LC50 value for rainbow trout exposed to HBCD was >6.8 ug/L (nominal) (>2.5 ug/L mean measured concentration), the highest concentration tested and twice HBCD's water solubility (3.4 ug/L). Based on the mortality and observation data, the 96-hour no morality concentration and the no-oberved-effect-concentration were 6.8 ug/L (nominal) (2.5 ug/L mean measured concentration) and was higher than the water solubility of HBCD.

Data Quality

Reliability: High

Data Reliability Remarks

This study was performed according to current guidelines and Good Laboratory Practices by a laboratory with considerable experience with these studies. Extensive attention was paid to analytical method development and performance.

Reference

>> Remarks

Graves, W and Swigert, J. (1997) Hexabromocyclododecane (HBCD): A 96-Hour Flow-Through Acute Toxicity Test with the Rainbow Trout (Oncorhynchus mykiss). Project Number: 439A-101. Wildlife International LTD, Easton, MD.

General

Study sponsored by the Chemical Manufacturers Association Brominated Flame Retardant Industry Panel, Arlington, VA.

EPA High Production Volume (HPV) Track

Ecotoxicity End Point:
Acute Toxicity to Aquatic Invertebrates

Sponsor ID	100921	Albemarle Corporation	Create Date	4/6/01
CAS Number	3194-55-6	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	100912	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	N

Revision Date: 12/5/01

Test Substance

Remarks: The test article was a composite of equal parts of the commercial hexabromocyclododecane (HBCD) commercial product produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. The test article composite was analyzed for characterization and homogeneity. The results of the analysis indicated the test article was homogeneous and contained the following components: HBCD beta isomer 8.5%, HBCD alpha isomer 6.0%, HBCD gamma isomer 79.1%.

Chemical Category

Method

>> Method/Guideline followed

OECD Method 202; TSCA Title 40 CFR, Part 797, Section 1300

>> Test Type

flow-through

>> GLP Yes

>> Year study performed 1997

>> Species

Daphnia magna

>> Analytical monitoring HPLC; Limit of Quantitation=0.4 ug/L

>> Exposure period 48 Hours

>> Statistical Method None - no dose response pattern

Remarks for Method

Daphnids were exposed to one of five test concentrations, a solvent control or the negative (well water) control. Two replicate test chambers were maintained for each treatment and control group. Ten daphnids were used in each test chamber for a total of 20 daphnids per test concentration. Nominal test concentrations were based upon the solubility of the test substance in water (3.4 ug/L) and the results of an exploratory rangefinding toxicity test. Nominal test concentrations were 1.5, 2.2, 3.2, 4.6 and 6.8 ug/L. Mean measured test concentrations were analytically determined (HPLC with UV/VIS detector) from samples of test water collected from each treatment and control group at the beginning and end of the test.

Results

Delivery of the test substance was initiated approximately 4 days prior to the introduction of the daphnids to the test water in order to acheive equilbrium of the test substance in the test chambers. Daphnids were indiscriminately assigned to exposure chambers at test initiation. Observations of mortality/immobility and other clinical signs were made approximately 2, 24 and 48 hours after test initiation. Cummulative percent mortality and immobility observed in the treatment groups were used to estimate EC50 values at 24 and 48 hours. The no mortality/immobility concentration and the no-observed-effect concentration (NOEC) were determined by visual interpretation of the mortality, immobility and clinical observation data.

Daphnid neonates used in the test were less than 24 hours old and were obtained from cultures maintained by Wildlife International Ltd, Easton, MD. Adult daphnids were cultured in water from the same source and at approximately the same tempreature as that used during the test except supplemented with selenium. Daphnids in the cultures were held for 15-29 days prior to collection of the juveniles for testing. The progency of 7 adults were used in the test. The adults were fed prior to test initiation, but neonates were not fed during the test. During the 14-day holding period preceeding the test, water temperatures ranged from 20.2 to 21.4 degrees C. The pH of the water ranged from 8.0 to 8.5. Disolved oxygen ranged from 8.2 to 9.0 mg/L.

A continuous-flow diluter was used to deliver each concentration of the test substance, a solvent control, and a negative (dilution water) control. Syringe pumps (Harvard Apparatus) were used to deliver the five test substance stock solutions and the solvent for the solvent control into mixing chambers assigned to each treatment level and the solvent control. The stock solutions were diluted with well water in the mixing chambers in order to obtain the desired test concentrations. The flow of dilution water to the mixing chambers was controlled by rotameters. Rotameters were calibrated prior to test initiaiton. The flow of test water from each mixing chamber was split and allowed to flow into replicate test chambers. The proportion of test water that was split into each replicate was checked prior to the test to ensure that flow rates varied by no more than +/- 10% of the mean for the two replicates.

The diluter was adjusted so that each test chamber received ~14 volume additions of test water every 24 hours. The stock solution delivery pumps were calibrated before the test, and the general operation of the diluter was checked visually at least two times daily during the test and once at the end of the test.

Test compartments were constructed from 300 mL glass beakers ~ 8 cm in diameter and 13 cm in height. The beakers were suspended in 8-L stainless steel test chambers filled with ~6.5 L of test water. Test chambers were indiscriminately positioned in a temperature-controlled water bath designed to maintain a temperature of 20+/-1 degreeC. The water bath was enclosed in a plexiglass ventilation hood. Test chambers were labeled with the project number, test concentration, and replicate.

The water used for culturing and testing was freshwater obtained from a well ~45 meters deep located on the Wildlife International Ltd. Site. The well water is characterized as moderately-hard water. The dissolved oxygen content of the water ranged from 8.8-8.9, 9.0-9.1, and 8.8-8.9 mg/L at 0, 24, and 48 hours, respectively. The pH of the water was 8.1, 8.2-8.4, and 8.2-

8.3 at 0, 24 and 48 hours, respectively. The temperature of the water ranged from 19.8-19.9 and 19.9-20.0 at 0 and 48 hours, respectively. The 0-hour dilution water measurements for hardness, alkalinity and specific conductance were 132 mg/L as CaC03, 176 mg/L as CaC03 and 320 umhos/cm, respectively.

Lighting was provided by fluorescent tubes that emitted wavelengths similar to natural sunlight. A photoperiod of 16 hours of light and 8 hours of darkness was controlled with an automatic timer. Light intensity at test initiation was ~ 242 lux at the surface of the water.

>> Nominal concentration 0, 0.0015, 0.0022, 0.0032, 0.0046, 0.0068 mg/L

>> Measured concentration 0, 0.0024, 0.0018, 0.0021, 0.0023, 0.0032 mg/L

>> Precision >

>> Endpoint Type LC0

>> Endpoint Value 0 **>> Unit used** mg/L

>> Concentration Type Nominal **>> Endpoint Time** 48

>> Statistical results

Statistics not performed due to lack of dose repsonse.

Results Remark

The selection of exposure concentrations took into consideration the water solubility limit (3.4 ug/L) and a finding of no acute toxicity from an exploratory range finding test. However, there was a potential to have a slight enhancement of HBCD's water solubility due to the use of dimethyl formamide (DMF) as a vehicle in the diluter system. For this reason, the highest test concentration seleceted was twice the defined solubility limit (i.e., 6.8 ug/L). The series of nominal test concentrations bracketed the solubility limit of HBCD by five concentrations.

Two sets of pretest samples were collected from the highest and lowest test concentrations and analyzed. The Day -3 and -2 samples indicated that the test concentrations were stable, but somewhat lower than expected. Measurements of HBCD concentration in all test chambers were made at the beginning and end of the test. These measurements indicated that HBCD concentrations were generally similar across all treatment levels, and may reflect a

phenomenon in the delivery system whereby HBCD adsorbed to the physical surfaces of the diluter system. This could be due to the hydrophobic nature of HBCD as evidenced by its nonpolar alkane structure and extremely low water solubility. This characteristic could have enabled the inert surfaces (e.g. Stainless steel and Teflon) of the diluter system to eventually become saturated with HBCD. As this process progressed, an equilibrium was established. The result of this new equilibrium was that concentrations of HBCD in the dilution water were approximately the solubility of HBCD in well water under flow-through conditions.

Dissolved oxygen concentrations of > or = 97% of saturation were observed throughout the test. Water pH ranged from 8.1-8.4. Total organic carbon in the dilution water at test initiation was <1.0 mg C/L.

Daily observations during the test showed that daphnids in the negative control and solvent control groups appeared healthy and normal. With the exception of one aberrant mortality in the 4.6 ug/L (nominal) treatment group, all daphnids in all treatment groups appeared normal throughout the test with no mortalities or overt signs of toxicity. Based on these results, EC50 values for 24 and 48 hours were estimated to be > 6.8 ug/L (nominal), the highest concentration tested.

Conclusions

HBCD was not acutely toxic to Daphnia magna. The 48-hour EC50 value for daphids exposed to HBCD was > 6.8 ug/L (nomimal) (>3.2 ug/L mean measured concentration), the highest concentration tested and twice HBCD's water solubility (3.4 ug/L). Based on the mortality, immobility and observation data, the 48-hour no mortality/immobility concentration and the no-observed-effect concentration was 6.8 ug/L (nominal) (3.2 ug/L mean measured concentration).

Data Quality

Reliability High

Data Reliability Remarks

This study was performed according to current guidelines and Good Laboratory Practices by a laboratory with considerable experience with these studies. Extensive attention was paid to analytical method development and performance.

Reference

>> Remarks

Graves, W and Swigert, J. (1997) Hexabromocyclododecane (HBCD): a 48-hour flow-through acute toxicity test with the cladoceren (Daphnia magna). Project Number: 439A-102. Wildlife International Ltd., Easton, MD.

General

EPA High Production Volume (HPV) Track

Ecotoxicity End Point:
Acute Toxicity to Aquatic Invertebrates

Sponsor ID	100921	Albemarle Corporation	Create Date	1/8/01
CAS Number	3194-55	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	N

Study sponsored by the Chemical Manufacturers Association Brominated Flame Retardant Industry Panel.

EPA High Production Volume (HPV) Track

Ecotoxicity End Point : Toxicity to Aquatic Plants

Sponsor ID	1100021	Albemarle Corporation	Create Date	4/4/01	
CAS Number	3194550	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1	
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	N	

Revision Date: 12/5/01

Test Substance

Remarks The test article was a composite of equal parts of the commercial hexabromocyclododecane (HBCD) commercial product produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. The test article composite was analyzed for characterization and homogeneity. The results of the analysis indicated the test article was homogeneous and contained the following components: HBCD beta isomer 8.5%, HBCD alpha isomer 6.0%, HBCD gamma isomer 79.1%.

Chemical Category

Method

>> Method/Guideline followed

OECD Method 201; TSCA Title 40, CFR, Part 797, Section 1050

>> Test Type

static

>> GLP Yes

>> Year study performed 1997

>> Species

Selenastrum capricornutum

>> End Point Cell densitities and area under the growth curve.

>> Analytical monitoring HPLC/UV/VIS Detector; LOQ=0.400 ug/L

>> Exposure period 96 Hours

>> Statistical Method Shapiro Wilk's;Bartlett's;Dunnett's;Bonferroni's t

Remarks for Method

The freshwater alga, Selenastrum capricornutum, was exposed to one of five test concentrations, a solvent control (DMF) or the negative (culture medium) control under static conditions for 96 hours. Three replicate test chambers were maintained for each treatment and control group. Nominal test concentrations were based on the solubility of the test substance in water (3.4 ug/L) and the results of an exploratory range finding toxity test. Nominal test concentrations were 1.5, 2.2, 3.2, 4.6 and 6.8 ug HBCD/L. The highest dose tested was also confirmed analytically (HPLC with UV/VIS

detector).

Test solutions were inoculated with 1.0 mL of an inoculum with an approximate (~) density of 1.0 x 10E6 cells/mL to achieve a final cell density of 1.0 x 10E4 cells/mL. Samples of the test solutions were collected from each replicate test chamber at ~24 hour intervals during the test to determine cell density. Cell densities and area under the growth curve values were determined for each replicate and were used to calculate % inhibition values relative to the controls over the 96-hour exposure period. EC10, EC50 adn EC90 values were calculated, if possible, based on cell densities and area under the growth curve values for each 24-hour interval. The no-observed-effect concentration (NOEC) was determined based on statistitical evaluation of the cell densities and area under the growth curve values.

A primary stock solution was prepared by dissolving HBCD in dimethylformamide (DMF). The concentration of the stock was 0.068 mg HBCD/mL. Stock concentrations and the resultant test concentrations were prepared on a total product basis. A solvent control was prepared by diluting 250 uL DMF to 2.5 L with culture medium to yield a solvent concentration equvalent ot that in the treatment groups.

Original cultures of the freshwater algae, Selenastrum capricornutum, were obtained from UTEX - The Culture Collection of Algae at the University of Texas at Austin, and have been maintained in culture medium at Wildlife International Ltd, Easton, MD. Algal cells used in this test were obtained from Wildlife International Ltd cultures that had been actively growing in culture medium for a least two weeks prior to test initiation. The control organisms were expected to exhibit exponential growth over the 96-hour exposure period. Exponential growth phase, defined as the period of growth where the algal cells are dividing at a constant rate, is indicated by the liner section of the growth curve.

The algal cells were cultured and tested in freshwater algal medium. Test chambers were sterile 250-ml Erlenmeyer flasks plugged with foam stoppers, and containing 100 mL of test or control algal medium. The test chambers were shaken continuously at 100 rpm, and held in an environmental chamber at 24 +/- 2 degrees C. Cool-white fluorescent lighting was used throughout the test (4310 +/- 431 lux). Samples of ~ 2 mL were collected from each treatment and control vessel at ~ 24 hour intervals during the 96-hour exposure. Cell counts were performed using an electronic particle counter (Coulter Electronics, Inc.). Samples of the test medium (test samples) were collected from each treatment and control group at the beginning and end of the test to measure concentrations of the test substance.

Results

>> Nominal concentration	0, 0.0015, 0.0022, 0.0032, 0.0046, 0.0068 mg/L
>> Measured concentration	Negative control, Solvent control, and 0.0037 mg/L
>> Precision	>

EPA High Production Volume (HPV) Track

Ecotoxicity End Point : Toxicity to Aquatic Plants

Sponsor ID	1102021	Albemarle Corporation	Create Date	4/3/01	
CAS Number	3194-55	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1	
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	N	

>> Endpoint Type EC0

>> Endpoint Value 0 **>> Unit used** mg/L

>> Concentration Type Nominal **>> Endpoint Time** 96

>> NOEC Precision > **>> NOEC** 0 **>> Unit used** mg/L

>> NOEC Concentration Type Nominal

>> NOEC Effect(s) assesse cell densisty and growth

>> LOEC Precision > **>> LOEC** 0 **>> Unit used** mg/L

>> LOEC Concentration Type Nominal

>> LOEC Effect(s) assesse cell density and growth

>> Response of Control Group (was it satisfactory? Yes

>> Statistical results

No statistically significant differences (p>0.05) were found between control and treated groups. Algal growth was not inhibited by HBCD, and EC10, EC50 or EC90 values could not be defined.

Results Remark

HBCD's 96 hr effect concentration in the freswater algae tested could not be determined. The NOEC is greater than HBCD's water solubility.

The mean measured concentration at the 6.8 ug/L dose level was 3.7 ug/L.

Conclusions

HBCD's 96 hr effect concentration in the freswater algae tested could not be determined. The NOEC is greater than HBCD's water solubility.

Data Quality

Reliability High

Data Reliability Remarks

EPA High Production Volume (HPV) Track

Ecotoxicity End Point : Toxicity to Aquatic Plants

Sponsor ID	1100[illegible]	Albemarle Corporation	Create Date	4/5/01
CAS Number	3194-55-6	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	113-012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	N

This study was performed according to current guidelines and Good Laboratory Practices by a laboratory with considerable experience with these studies. Extensive attention was paid to analytical method development and performance.

Reference

>> Remarks

C. Roberts and J. Swigert. Hexabromocyclododecane (HBCD) A 96-Hour Toxicity Test with the Freashwater Alga (Selenastrum capricornutum). Wildlife International Ltd. Project Number: 439A-103. June 3, 1997. Wildlife International Ltd., Easton, MD.

General

Sponsor ID	110002	Albemarle Corporation	Create Date	4/2001	
CAS Number	3194557	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	?	
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	N	

Revision Date: 12/5/01

Test Substance

Remarks A sample of the hexabromocyclododecane (HBCD) commercial product was obtained from one manufacturer, Great Lakes Chemicals Corporation (West Lafaytette, IN).

Chemical Category

Method

>> Method/Guideline followed

Not specified.

>> Test Type

Not specified.

>> GLP Unknown

>> Year study performed 1987

>> Species

Skeletonema costatum, Thalassionsira pseudonana, Chlorella sp.

>> End Point cell numbers

>> Analytical monitoring Capillary column GLC; DL not specified.

>> Exposure period 72 Hr S. Costatum, T. Pseudonana; 96 Hr Chlorella

>> Statistical Method None - used linear regression to determine EC50

Remarks for Method

Each test was replicated. Population density was estimated by cell counts on a hemacytometer. The test article was introduced into growth flasks by adding 0.05 ml test article in acetone to 51 ml growth medium with algae. Algal species tested were S. costatum (Greville) Cleve, T. pseudonana Hasle and Heindal, and Clorella sp., and were obtained from University of Rhode Island, Woods Hole Oceanographic Institution, and the Culture Collection of Algae, University of Texas at Austin, respectively. Growth media were prepared from seawater collected from an inshore site on the Gulf of Mexico and from five commercial sea salt formulations. Toxicity was expressed as the EC50 based on the cell numbers after incubation for 72 (S. costatum) or 96 hrs (T. pseudonana, Chlorella

sp.). The EC50 was derived by straight line graphical interpolation without calculation of confidence intervals. The highest test article concentration was determined by adding the test article slowly to growth media and observing the highest concentration at which crystals did not form.

Results

>> Nominal concentration

>> Measured concentration

>> Precision

>> Endpoint Type

>> Endpoint Value 0 **>> Unit used**

>> Concentration Type **>> Endpoint Time** 0

>> NOEC Precision **>> NOEC** 0 **>> Unit used**

>> NOEC Concentration Type

>> NOEC Effect(s) assesse

>> LOEC Precision **>> LOEC** 0 **>> Unit used**

>> LOEC Concentration Type

>> LOEC Effect(s) assesse

>> Response of Control Group (was it satisfactory?

>> Statistical results

Results Remark

EPA High Production Volume (HPV) Track

Ecotoxicity End Point : Toxicity to Aquatic Plants

Sponsor ID	1100021	Albemarle Corporation	Create Date	4/5/01	
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	2	
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	N	

Growth of Chlorella sp. was not inhibited by HBCD at the highest dose tested, 1.5 mg/L. HBCD's EC50 for S. costatum ranged from 9.0-12.0 ug/L in the six media. Similarly, HBCD's EC50 in T. pseudonana ranged from 0.05-0.37 mg/L in the six media.

The pH of the six different growth media ranged from 7.6-8.2. No relationship of pH to toxicity was found for HBCD.

There was little variation in the response of S. costatum to HBCD among the media, but the response to T. pseudonana varied widely. S. costatum may be more sensistive to HBCD than T. psuedonana.

Conclusions

HBCD's 96 hour EC50 in Chlorella sp., tested in 6 different growth media, was > 1.5 mg/L. HBCD's 72 hour EC50 in S. Costatum and T. Pseudonana in 6 different growth media ranged from 0.009-0.012 and 0.5-0.36 mg/L, respectively. All EC50 values determined in the three marine algae were greater than HBCD's water solubility (0.0034 mg/L).

Data Quality

Reliability good

Data Reliability Remarks

Reference

>> Remarks

Walsh, G., Yoder, M., Mclaughlin, L., Lores, E. (1987) Responses of marine unicellular algae to brominated organic compounds in six growth media. Ecotoxicology and Environmental Safety, 14, 215-222.

General

EPA High Production Volume (HPV) Track

Toxicity End Point:
Acute Toxicity

Sponsor ID	100021	Albemarle Corporation	Create Date	3/6/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y

Revision Date:
4/10/01

Test Substance

Remarks: A form of hexabromocyclododecane (HBCD) supplied as test article by Saytech Inc. No further details are available.

Chemical Category

Method

>> Method/Guideline followed

Not known.

>> GLP Unknown

>> Year study performed 1978

>> Species

rat

>> Strain no data

>> Sex Both

>> Number of males per dose 5

>> Number of females per dose 5

>> Vehicle Corn oil

>> Route of Administration

Oral

Remarks for Method

Albino rats in groups of ten (5M:5F), 192-260 g, were administered a single dose (10 g/kg) orally and observed for 14 days. The highest volume used was 40 ml/kg. The vehicle was corn oil

Results

>> Precision >

>>Acute Lethal Value 10000

>> Unit mg/kg-bw

>> Deaths per Dose

One of five males died on test. No females died on test.

Results Remark

Conclusions

The oral LD50 of HBCD in the rat was > 10,000 mg/kg body weight.

Data Quality

Reliability Acceptable

Data Reliability Remarks

Sponsor ID	1100021	Albemarle Corporation	Create Date	12/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y

This study is old and not performed according to current guidelines. Nonetheless, the results are consistent with the general lack of toxicity associated with this material in other mammalian studies. Thus, it was found acceptable.

Reference

>> Remarks

Lewis, C and Palanker, A. (1978) Final Report. Oral LD50 (Rat). Experiment Reference No.: 78385-1. Consumer Product Testing Company Incorporated, Fairfield, NJ.

General

Sponsored by Saytech, Inc., Sayreville, NJ.

EPA High Production Volume (HPV) Track

Toxicity End Point:
Acute Toxicity

Sponsor ID	[illegible]	Albemarle Corporation	Create Date	[illegible]	
CAS Number	[illegible]	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	[illegible]	
Consortia ID	[illegible]	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed	Y	

Revision Date:
12/5/01

Test Substance

Remarks: A form of hexabromocyclododecane (HBCD) supplied as test article by Saytech Inc. No further details are available.

Chemical Category

Method

>> Method/Guideline followed

Not known.

>> GLP Unknown

>> Year study performed 1978

>> Species

rabbit

>> Strain New Zealand White

>> Sex Both

>> Number of males per dose 3

>> Number of females per dose 3

>> Vehicle None

>> Route of Administration

Dermal

Remarks for Method

Sponsor ID	1100021	Albemarle Corporation	Create Date	3/6/01	
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	2	
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed	Y	

Method was described as that of Hagen (1959). Albino rabbits in groups of six (3M:3F), one half with abraded skin, 1.88-2.07 kg, highest dose level mechanically possible, single application dermally under occluded patch, observed for 14 days. Material used as received. Upper limit possible due to mechanical and physical limitations is 8 g/kg body weight.

Results

>> Precision >

>>Acute Lethal Value 8000

>> Unit mg/kg-bw

>> Deaths per Dose

No animals died on test.

Results Remark

Conclusions

The dermal LD50 of HBCD in rabbits was > 8,000 mg/kg body weight.

Data Quality

Reliability Acceptable.

Data Reliability Remarks

This study is old and not performed according to current guidelines. Nonetheless, the results are consistent with the general lack of toxicity associated with this material in other mammalian studies. Thus, it was found acceptable.

Reference

>> Remarks

Lewis, C and Palanker, A. (1978) Final Report. Dermal LD50 (Rabbit). Experiment Reference No.: 78385-2. Consumer Product Testing Company Incorporated, Fairfield, NJ.

General

Sponsored by Saytech, Inc., Sayreville, NJ.

EPA High Production Volume (HPV) Track

Toxicity End Point:
Acute Toxicity

Sponsor ID	1100021	Albemarle Corporation	Create Date	10/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	3
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y

Revision Date:
4/11/01

Test Substance

Remarks: A form of hexabromocyclododecane (HBCD) supplied as test article by Saytech Inc. No further details are available.

Chemical Category

Method

>> Method/Guideline followed

Not known.

>> GLP: Unknown

>> Year study performed: 1978

>> Species

rat

>> Strain: no data

>> Sex: Both

>> Number of males per dose: 5

>> Number of females per dose: 5

>> Vehicle: None

>> Route of Administration

Inhalation

Remarks for Method

Sponsor ID	1100621	Albemarle Corporation	Create Date	1/6/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y

Albino rats in groups of 10 (5M:5F), 233-292 g, exposed to concentrations of 200 mg/L (highest possible chamber concentration) for one hour, observed two weeks. Material used as received.

Results

>> Precision >

>>Acute Lethal Value 200

>> Unit mg/L(air)

>> Deaths per Dose

No animals died on test.

Results Remark

Conclusions

The inhalation LC50 of HBCD in rats was > 200 mg/L for a 1 hour exposure.

Data Quality

Reliability Acceptable.

Data Reliability Remarks

Sponsor ID	100021	Albemarle Corporation	Create Date	1-01	
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	3	
Consortia ID	101C12	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y	

This study is old and not performed according to current guidelines. Nonetheless, the results are consistent with the general lack of toxicity associated with this material in other mammalian studies. Thus, it was found acceptable.

Reference

>> Remarks

Lewis, C and Palanker, A. (1978) Final Report. Inhalation LC50 (Rat). Experiment Reference No.: 78385-2. Consumer Product Testing Company Incorporated, Fairfield, NJ.

General

Sponsored by Saytech, Inc., Sayreville, NJ.

EPA High Production Volume (HPV) Track

Toxicity End Point:
Developmental Toxicity/Teratogenicity

Sponsor ID	1100021	Albemarle Corporation	Create Date	1/6/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9 10-hexabromo-	Study Number	1
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed	

Revision Date: 12/5/01

Test Substance

Remarks: The test article was a composite of equal parts of the commercial hexabromocyclododecane (HBCD) commercial product produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. The test article composite was analyzed for characterization and homogeneity. The results of the analysis indicated the test article was homogeneous and contained the following components: HBCD beta isomer 8.5%, HBCD alpha isomer 6.0%, HBCD gamma isomer 79.1%.

Chemical Category

Method

>> Method/Guideline followed: EPA OPPTS Method 870.3700; OECD 414

>> GLP: Yes

>> Year study performed: 1999

>> Species: rat

>> Strain: **Mammal strai:** Sprague-Dawley

>> Sex: F

>> Number of males per dose: 0

>> Number of females per dose: 25

>> Route of Administration: Oral

>> Days of Gestation: 6-19

>> Frequency of treatment: Once daily

>> Doses: 0, 250, 500, 1000 mg/kg body weight

>> Control Group: Yes **Concurrent control**

>> Statistical Method: See Remarks for Method.

Remarks for Method

Sponsor ID	1100021	Albemarle Corporation	Create Date	1/6/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	

Hexabromocyclododecane (HBCD) was administered by gavage in corn oil to three groups of 25 bred Crl:CD(SD)IGS BR (Charles River Laboratories, Raleigh, NC) rats once daily from gestation days 6 through 19. Dosage levels were 250, 500 and 1000 mg/kg/day administered in a dose volume of 5 ml/kg. A concurrent control group composed of 25 bred females received the vehicle, corn oil, on a comparable regimen. Clinical observations, body weights and food consumption were recorded. On gestation day 20, a laparohysterectomy was performed on all animals. The uteri and ovaries were examined and the numbers of fetuses, early and late resorptions, total implantations and corpora lutea were recorded. Mean gravid uterine weights and net body weight changes were calculated for each group. The fetuses were weighed, sexed and examined for external soft tissue and skeletal malformations and variations.

Appropriate statistical tests were used for each end point and included a one-way ANOVA with Dunnet's test, and Kruskal Wallis test with Mann-Whitney U test.

Results

>> Maternal Precision/NOAEL =

>> Maternal NOAEL dose 1000 **>> Unit used** mg/kg-bw

>> Maternal NOAEL effect None

>> Maternal Precision/LOAEL >

>> Maternal LOAEL dose 1000 **>> Unit used** mg/kg-bw

>> Maternal LOAEL effect None

>> Developmental Precision/NOAEL =

>> Developmental NOAEL dose 1000 **>> Unit used** mg/kg-bw

>> Developmental NOAEL effect None

>> Developmental Precision/NOAEL >

>> Developmental LOAEL dose 1000 **>> Unit used** mg/kg-bw

>> Developmental LOAEL effect None

>> Actual dose

As given above.

>> Maternal data with dose level (with NOAEL value).

No adverse effects detected.

>> Fetal data with dose level (with NOAEL value).

No adverse detected.

>> Statistical results

See Methods.

Results Remark

All maternal animals survived to the scheduled necropsy on gestation day 20. One female in the 500 mg/kg/day group delivered on gestation day 20 and was examined at the scheduled laparohysterectomy. No treatment-related clinical signs were observed at any dose level. Body weight gain and food consumption were not adversely affected at any dose level. At necropsy, no treatment-related findings were observed. Intrauterine growth and survival were unaffected by test article administration at any dose level. No treatment-related fetal malformations or developmental variations were observed in any of the treated groups.

Conclusions

The no-observed-adverse-effect level for maternal toxicity and developmental toxicity was 1000 mg HBCD/kg/day administered on days 6-19 of gestation.

Data Quality

Reliability High

Data Reliability Remarks

This study was performed according to current guidelines for repeated dose studies under Good Laboratory Practices by a laboratory experienced in the performance of studies of this type.

Reference

>> Remarks

Stump, D. (1999) A Prenatal Developmental Toxicity Study of Hexabromocyclododecane (HBCD) in Rats. Laboratory Study No.: WIL-186009. WIL Research Laboratories, Inc., Ashland, OH.

General

Sponsored by Chemical Manufacturers Association Brominated Flame Retardant Industry Panel.

EPA High Production Volume (HPV) Track

Toxicity End Point:
Developmental Toxicity/Teratogenicity

Sponsor ID	1102021	Albemarle Corporation	Create Date	1/6/01	
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	2	
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:		

Revision Date: 12/5/01

Test Substance

Remarks The test article was manufactured by Daiichi Kogyo Seiyaku K.K. No further information on its composition is known.

Chemical Category

Method

>> Method/Guideline followed

Not specified.

>> GLP Unknown

>> Year study performed 1985

>> Species

rat

>> Strain **Mammal strai** Wistar

>> Sex F

>> Number of males per dose 0 **>> Number of females per dose** 20

>> Route of Administration Oral

>> Days of Gestation 0-20

>> Frequency of treatment Daily

>> Doses 0, 0.01, 0.1 and 1% of the Diet

>> Control Group Yes **Concurrent control**

>> Statistical Method

Not specified.

Remarks for Method

The Murai et al study consisted of a 7 day dose range finding study (n=5 rats/dose group) and a combined teratogenicity-developmental study (n=20/dose group). Doses in the 7 day range finding study were 0, 0.3, 1, 3 or 10 g/kg/day. Doses as high as 10 g/kg/day produced no evidence of toxicity. A statistically significant (P<0.01) increase in liver weight was noted in groups receiving > 1 g/kg/day. Doses for the combined teratogenicity-developmental study were based on this increase in liver weight.

In the combined teratogenicity-developmental study, pregnant female rats were fed diets containing 0, 0.01, 0.1, or 1% HBCD on days 0-20 of gestation. Daily doses were estimated by the authors to be 0, 5, 50 or 500 mg/kg/day and the average total dose/rat/group was estimated to be 0, 0.13, 1.28 or 12.0 g/kg. Rats were observed daily and body weight and food consumption measured. Fourteen rats from each group were sacrificed on day 20 of gestation and their fetuses were examined for toxicity or teratogenicity. Approximately 150 fetuses/dose level were examined for evidence of teratogenicity. All fetuses from all litters were examined for signs of external anomalies. Approximately 2/3 of the fetuses/dam were examined for skeletal abnormalities; the remaining fetuses from each dam were examined for any abnormalities of the internal organs. In addition, six rats from each group were allowed to deliver their litters and growth of the litters was observed until the 7th week post-parturition.

Results

>> Maternal Precision/NOAEL >

>> Maternal NOAEL dose 1000 **>> Unit used** mg/kg in feed

>> Maternal NOAEL effect No adverse effects, increased liver wt at 1% dose.

>> Maternal Precision/LOAEL >

>> Maternal LOAEL dose 1000 **>> Unit used** mg/kg in feed

>> Maternal LOAEL effect No adverse effecs.

>> Developmental Precision/NOAEL >

>> Developmental NOAEL dose 1000 **>> Unit used** mg/kg in feed

>> Developmental NOAEL effect No adverse effects.

>> Developmental Precision/NOAEL >

>> Developmental LOAEL dose 1000 **>> Unit used** mg/kg in feed

>> Developmental LOAEL effect No adverse effects.

>> Actual dose

Estimated as 0, 5, 50, 500 mg HBCD /kg bd wt/day

>> Maternal data with dose level (with NOAEL value).

Only effect detected in dams was an increase in liver weight at the 1% dose level.

>> Fetal data with dose level (with NOAEL value).

No effects detected in fetuses.

>> Statistical results

See results remarks.

Results Remark

The authors' estimated the doses in the feed were equivalent to 0, 5, 50 or 500 mg HBCD /kg body weight /day. No adverse effects were detected in any treatment group with respect to maternal weight gain, food consumption, or gross apprearance of internal organs. The mean liver (absolute and relative to body weight) weight in the 1% group was statistically different (higher) from the control mean. Normal development was seen in neonates carried through to six weeks of age.

There was no adverse effect of treatment on the number of corpera lutea, implants, resporptions, live fetuses, sex ratio, or body or placental weight. No fetal deaths occured in any group. No external, skeletal or viseral malformations were detected. A few skeletal variations were detected but where of similar types and numbers in the control and treated groups.

There was no significant differences between the control and treated groups in the number of implantation, live newborns, dead newborns, live newborn parturition index. The weaning and survival index was comparable in the control and treated groups. Body weight chnages in the newborns was comparable in all groups.

Conclusions

No reproductive or developmental effects where detected in rats at HBCD doses up to 1% in the diet (~500 mg/kg/d) administered from days 0-20 of gestation. Further, normal development was seen in neonates carried through to six weeks of age.

Dose levels: 0, 0.01, 0.1, or 1% HBCD on days 0-20 of gestation [Murai estimate: 0, 5, 50 or 500 mg/kg/day]. No teratogenic effects. Normal development in neonates carried through age 6 wks. NOEL = 1% of diet.

Data Quality

Reliability Good.

Data Reliability Remarks

One author of this study was associated with the National Institute of Hygienic Science, Osaka branch.

Reference

>> Remarks

Murai, T. Kawasaki, H., Kanoh, S. 1985. Studies on the tosicity of insecticides and food additives in pregnant rats - Fetal toxicity of Hexabromocyclododecane. Pharmacometrics (Japan) 29(6):981-986.

General

Funding for this study was provided by Japan's Ministry of Health and Welfare.

Sponsor ID	110021	Albemarle Corporation		Create Date	4/6/01
CAS Number	3194-55-6	Cyclododecane, 1,2,5,6,9,10-hexabromo-		Study Number	
Consortia ID	101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)		Completed	

Revision Date: 12/5/01

Test Substance

Remarks: The test article was a composite of equal parts of the commercial hexabromocyclododecane (HBCD) commercial product produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. The test article composite was analyzed for characterization and homogeneity. The results of the analysis indicated the test article was homogeneous and contained the following components: HBCD beta isomer 8.5%, HBCD alpha isomer 6.0%, HBCD gamma isomer 79.1%.

Chemical Category

Method

>> Method/Guideline followed

OECD Method 407

>> GLP Yes **>> Year study performed** 1997

Were Good Laboratory Practices followed in the st

>> Species

rat

>> Strain Mammal strai Sprague-Dawley

>> Sex Both

>> Number of males per dose 6 **>> Number of females per dose** 6

>> Route of Administration Oral

>> Exposure Period 28

Duration of study in days (for example, 28 days, 90 d

>> Frequency of treatment Once per day

Number of doses per day, week, etc. This is particularly relevant for inhalation experiments -- 6hrs/day, 5 d

>>Doses 0, 125, 350, 1000 mg/kg/day; dosage volume=5 ml/kg

List all doses used in te

>> Control Group Yes

Concurrent contro

>> Post observation period 14 Days

Length of time animals observed after last d

>> Statistical Method See Remarks for Method section.

Cite statistical methods use

Remarks for Method

Results

Hexabromocyclododecane (HBCD) was administed orally by gavage in corn oil to three groups of Sprague-Dawley Crl:CD BR (Charles River Laboratories, Inc., Portage, MI) rats for a period of 28 consecutive days at doses of 125, 350 or 1000 mg/kg/day administered in a dosage volume of 5 ml/kg. The test groups consisted of 6 males and 6 females in the 125 and 350 mg/kg/day groups, and 12 males and 12 females in the 1000 mg/kg/day group. A concurrent control group (n=12 males and females) was treated in a similar manner with the vehicle, corn oil. At the end of the dosing period, 6 animals/sex/group were euthanized and necropsied. The remaining 6 animals/sex in the control and 1000 mg/kg/day groups remained on test untreated for a 14 day recovery period. At the end of the recovery peroid, all animals were euthanized and necropsied. Animals were 6 weeks of age at study initiation.

Animals were observed twice daily for mortality and morbundity. Clinical signs were recorded daily. Body weights and food consumption were measured weekly. Functional observational battery and motor activity evaluations were performed during weeks 1 (pretest), 3, and 5 (recovery). Samples for hematology and serum chemistry evaluations were collected at the primary (28 day) and recovery (42 day) necropsies. Complete necropsies were performed on all rats. The brain, liver, kidney, heart, spleen, testes and epidymus or ovaries, adrenal glands, and thymus from all animals were weighted at each necropsy. Approximately 40 tissues were

collected and preserved at each necropsy from each animal. The following tissues were examined microscopically from the control and high dose animals: liver, kidney, heart, spleen, testes (males), prostate (males), seminal vesices (males), epididymus (males), ovaries (females), adrenal glands, thymus, bone with marrrow (sternebra), brain, stomach, cecum, duodenum, ileum, jejunum, lymph node, peripheral nerve (sciatic), spinal cord, lung, trachea, uterus (females), urinary bladder, and all gross lesions. The lungs, liver, kidney, stomach, gross lesions and target organs were examined in all dose levels.

Body weights, weight gain, food consumption, functional observation battery and motor activity results of treated animals were compared statistically by sex and treatment day to their respective control groups ($p<0.05$ or <0.01).

Concentrations of the dosing suspensions were confirmed. Homogeneity determinations were performed on study days 0, 13, and 27.

All statistical analyses were conducted using two-tailed tests for minimum significance levels of 1% and 5% comparing the treatment groups to the vehicle control group by sex. Analysis of body weight change, food consumption, clinical pathology values, continuous functional observational battery data and absolute and relative organ weight data were analyzed with a one-way analysis of variance followed by Dunntt's test. Discontinuous (ordinal or descriptive) functional observational battery data were analyzed using Fisher's exact test. Statistical tests for locomotor activity data were performed using SAS/STAT statistical software. Clinical laboratory values for cell types that occur at a low incidence (i.e., monocytes, eosinophils and basophils) were not subjected to statistical analysis.

>> NOAEL Precision >=

>> NOAEL dose 1000 **>> Unit** mg/kg-bw

>> NOAEL Effec

(e.g., decrease in body weight, organ Increase in liver weight in the absence of histopathlogic or clincal chemistry changes.

>> LOAEL Precision >

>> LOAEL dose 1000 **>> Unit** mg/kg-bw

>> LOAEL Effect
(e.g., decrease in body weight, organ

None noted.

>> Actual dose received by dose level by sex (if availabl

Test article administered by gavage.

>> Toxic response A brief narrative describing toxic response or effects, by dos

No evidence of toxicity was observed at any dose level.

>> Statistical results Note statistical results, with appropri ρ value

See Results Remarks section.

Results Remark Provide at a minimum qualitative descriptions of elements where dose effect related observations of elements were seen:

Survival was not affected by administration of the test article. All animals survived to the scheduled necropsy. Clinical signs observed during the study were nonspecific, low in incidence, non-dose-related, and not considered related to test article.

Body weights, weight gain and food consumption were not affected by treatment. No statistically significant differences in mean body weight between control and treated animals were detected with the exceptoin of an increase in mean femal body weight in the 350 mg/kg/day group during week 2. Mean female body weight at that time point was 196 g in the 350 mg/kg/day group vs. 179 g in the control group. No statistically significant differences in body weight gain between the control and treated animals with the expectation of a decrease in mean male body weight gain in the 1000 mg/kg/day recovery group during week 1 of recovery. Mean male body weight gain at that time point was 21 g vs 31 g in the control group; mean male body weight was not statistically different from the control mean. No statistically significant differences in food consumption between control and treated animals were detected with the exception of an increase in mean female food consumption in the 350 mg/kg/day during weeks, -1, 1, and 2 of treatment. Mean female food consumption at those time points were 18, 17 and 17 g vs. 16, 15 and 15 g in the control group, respectively.

Results of the functional observation battery and motor activity tests were not affected by treatment. No statistically significant differences were observed between the control and

treated animals at any time point ($p<0.05$).

No statistically significant differences between control and treated animals were found for hematology parameters with the exception of an increase in mean activated partial thromboplastin time in the 1000 mg/kg/day males on week 4 and a decrease in the mean prothrombin time in the 1000 mg/kg/day females on week 4. These statistical differences were not of toxicological significance.

No toxicologically significant effects on serum chemistry values related to test article administration were observed at the 28 day primary and 42 day recovery necropsies. Scattered instances of statistically significant differences between treated and control animals were detected for some serum chemistry parameters at the 28 day primary necropsy. These scattered statistical differences were not considered toxicologically significant because the statistical differences occurred in the absence of a dose response, in the absence of the accompanying clinical chemistry changes expected, in the opposite direction from what occurs in a toxic stae, in a direction which is without physiologic significance, or due to potential interference with the laboratory method. No statistically significant differences in serum chemistry parameters were detected between groups at the 42 day recovery necropsy.

No gross lesions attributable to test article administration were detected at either necropsy. Gross lesions were nonspecific, low in incidence, non-dose-related, and considered incidental.

No microscopic lesions attributable to test article administration were detected on histopatholgic exam. Microscopic changes were nonspecific, low in incidence, non-dose-related and considered incidental.

No statistically significant differences in organ weight or organ to body weight ratios were detected between control and treated animals with one exception. Absolute liver weights were statistically significantly increased with respect to control mean at the 28 day necropsy in males in the 1000 mg/kg/day group and in females in the 350 and 1000 mg/kg/day groups. Liver to body weight ratios in the 350 and 1000 mg/kg day male and female groups were statistically increased at the 28 day necropsy. At the recovery necropsy, male absolute and liver to body weight ratio were statistically comparable to the contol mean. Female absolute liver weight and liver to body weight ratio were statistically increased compared to the control mean. The difference in absolute liver weight between control and treated females was less pronounced at the end of the recovery period, indicating the increase in liver weight was reversible in females as well as males. In the absence of test article related histologic and serum chemistry changes, increases in liver weight are considered an adaptive rather than toxic response, are not uncommon in the rat, and are most likely the result microsomal

induction.

Conclusions

Input optional information or further explain the contents of a particular section, much as is done in the "Discussion" portion of a publication in academic journals.

No systemic toxicity was observed at any dose level. The No Observed Adverse Effect Level of HBCD administered orally to male and female rats for 28 consecutive days was > or = 1000 mg/kg/day, the highest dose tested.

Data Quality

Reliability High

Denote the reliability of data, at the discretion of the person preparing the robust su

Data Reliability Remarks **Add comments about how reliability of data was determined, or add re**

This study was performed according to current guidelines for repeated dose studies under Good Laboratory Practices by a laboratory experienced in the performance of studies of this type.

Reference

Cite the full reference for the critical study on which the robust study summary is based. List other appropriate references that support this summary and, have been reviewed for

>> Remarks

Chengelis, C. (1997) A 28-Day Repeated Dose Oral Toxicity Study of HBCD in Rats. Laboratory Study Number: WIL-186004. WIL Research Laboratories, Inc., Ashland, OH.

General

Add any statement that doesn't fit into any of the other f

Study sponsored by the Chemical Manufacturers Association Brominated Flame Retardant Industry Panel.

Sponsor ID	[redacted]	Albemarle Corporation	Create Date	1/6/01
CAS Number	[redacted]	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	2
Consortia ID	[redacted]	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed.	[redacted]

[redacted]

Revision Date: 12/5/01

Test Substance

Remarks: The test article was a composite of equal parts of the commercial hexabromocyclododecane (HBCD) commercial product produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. The test article composite was analyzed for characterization and homogeneity. The results of the analysis indicated the test article was homogeneous and contained the following components: HBCD beta isomer 8.5%, HBCD alpha isomer 6.0%, HBCD gamma isomer 79.1%.

Chemical Category

Method

>> Method/Guideline followed

OECDMethod 408, EPA OPPTS Method 870.3100

>> GLP Yes **>> Year study performed** 2001

Were Good Laboratory Practices followed in the st

>> Species

rat

>> Strain Mammal strai Sprague-Dawley

>> Sex Both

>> Number of males per dose 15 **>> Number of females per dose** 15

>> Route of Administration Oral

>> Exposure Period 90

Duration of study in days (for example, 28 days, 90 d

>> Frequency of treatment Once daily by gavage

Number of doses per day, week, etc. This is particularly relevant for inhalation experiments -- 6hrs/day, 5 d

>>Doses 0, 100, 300, 1000 mg/kg-bw; dose volume = 5 ml/kg

List all doses used in te

>> Control Group Yes

Concurrent contro

>> Post observation period 30 day recovery period

Length of time animals observed after last d

>> Statistical Method ANOVA, Dunnett's test, Others

Cite statistical methods use

Remarks for Method

Results

The test article, a composite of three lots of commercial hexabromocyclododecane (HBCD), was administered by oral gavage in corn oil once daily to four groups of Crl:CD(SD)IGS BR rats (n=15/sex/group) at dose levels of 0 (control), 100 (low), 300 (mid) and 1000 (high) mg/kg/day seven days per week for 90 days. The dosage volume was 5 ml/kg. The control animals received the vehicle, corn oil, only. At the end of the 90-day treatment period, 10 animals/sex/group were euthanized and necropsied. The remaining rats continued on test untreated for a 28-day recovery period prior to necropsy.

In addition to the main toxicology groups, two satellite groups of 20 animals/sex/group were treated concurrently in an identical manner at dose levels of 0 or 1000 mg HBCD/kg/day for up to 90 days. Body weights were recorded weekly. Two animals/sex/group were euthanized on study days 2, 6, 9, 13, 20, 27, 55, 89, 104 and 118, and blood and body fat (mesenteric and/or omental) were collected. The body fat was analyzed for HBCD content.
Animals in the main toxicology groups were observed twice daily throughout the study for mortality and morbidity. Body weights and food consumption were measured weekly. Blood was collected at study weeks 3 (n=5/sex/group), 13 (n=10/sex/group) and 17 (n=5/sex/group) for hematology, serum chemistry and hormone (T3, T4 and TSH) measurements. Urine was collected prior to each necropsy, at study weeks 13 and 17, for urinalysis. Ocular examinations were performed prior to study initiation and during study weeks 12 and 15.

Functional Observational Battery and Locomotor Activity evaluations were performed on 5 animals/sex/group prior to study initiation, during the last week of test article administration (study week 13), and during the recovery period. An examination of vaginal cytology (for estrus cycle determinations) was performed on study days 69-90. At each necropsy, sperm motility/viability, morphology, and number were assessed. Complete necropsies were performed on all animals. Approximately 40 organs or tissues/animal were collected and preserved. The adrenals, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus, thyroids with parathyroids, and uterus with cervix were weighed. Paraffin sections of tissues stained with hematoxylin and eosin from the control and 1000 mg/kg/day dose groups and the liver, lungs and thyroid glands in the 100 and 300 mg/kg/day doses, and gross lesions from all animals were examined under the light microscope. Livers from five randomly chosen animals/sex from the control and 1000 mg/kg/day dose groups were examined microscopically using Oil Red O or periodic acid Schiff's (PAS) reagent for evidence of lipid accumulation or glycogen accumulation/depletion, respectively. Statistical comparisons by sex and treatment day were made between the control and treated animals where indicated ($p<0.05$).

>> NOAEL Precision >=

>> NOAEL dose 1000 **>> Unit** mg/kg-bw

>> NOAEL Effec See Results Remarks.

(e.g., decrease in body weight, organ

>> LOAEL Precision >

>> LOAEL dose 1000 **>> Unit** mg/kg-bw

>> LOAEL Effect No adverse effects detected.

(e.g., decrease in body weight, organ

>> Actual dose received by dose level by sex (if availabl

As given under Doses.

>> Toxic response A brief narrative describing toxic response or effects, by dos

See Results Remarks.

>> Statistical results **Note statistical results, with appropri ρ value**

See Results Remarks.

Results Remark **Provide at a minimum qualitative descriptions of elements where dose effect related observations of elements were seen:**

No test article-related effect on mortality occurred. Clinical signs were non-specific, low in incidence, non-dose-related and not related to test article administration. No test article-related changes occurred in body weight, food consumption, Functional Observational Battery or Locomotor Activity. No test article-related effects on hematologic parameters were noted. No test article?related ocular lesions were detected at the ophthalmic exams. No test article-related changes were noted on the estrus cycle as determined by vaginal cytology, or on sperm motility/viability, morphology, and number. Instances of statistically significant differences between control and some treatment groups were detected at study week 13 in the clinical chemistry data, hormone data, organ weight data and histology findings. They were generally secondary to the inducing effects on the liver or were otherwise not considered adverse effects of treatment as discussed further below.

Statistically significant ($p<0.05$) test article-related clinical chemistry changes at week 13 include an increase in albumin (all dose levels for males), total protein (all dose levels for females and 1000 mg/kg/day for males), globulin (300 and 1000 mg/kg/day for females), and chloride (all doses for both sexes). In addition, increased gamma glutamyltransferase levels were noted in the 1000 mg/kg/day group ($p<0.05$). Thyroxine (T4) levels were decreased at study week 13 compared to the control mean in all male dose groups and the 300 and 1000 mg/kg/day dose females ($p<0.05$). There were no corresponding statistical effects on T3 and TSH. While potentially test article-related, the changes in serum chemistry parameters were not of sufficient magnitude to be adverse, occurred in otherwise clinically normal animals, tended to be within or close to historical control values, and were not present at the end of the recovery period; furthermore, these serum albumin and gamma glutamyltransferase increases were probably secondary to the increases in liver weight. The increases in serum chloride were probably secondary to be presence of free bromide in the test article preparation which interfered with the chloride determination methodology. The decrease in T4, which was also reversible, was also probably secondary to increased liver weight (secondary to microsomal enzyme induction, known to cause increased metabolism and clearance of T4 in the rat).

The incidence of observations noted at gross necropsy was low and there was no evidence of frank organ damage. On histopathologic examination of tissues, relatively mild findings occurred in both the control and treated groups. Potential test article?related histologic changes were identified in the liver and thyroid glands but these would not be considered indicative of frank toxicity. These organs were examined microscopically in all groups at both necropsies. The liver changes in male rats at the 90-day necropsy (Study Week 13) were characterized as minimal hepatocellular vacuolation and occurred in 10% of control males and ~50% of the males at 100, 300 and 1000 mg/kg/day. Minimal hepatocellular vacuolation was also detected in females in the control and test article treated groups without a clear dose response (3 to 4/10 animals per group) but, mild and moderate vacuolation was detected in females only in the 300 (1/10) and 1000 mg/kg/day (2/10) dose groups. Minimal to mild hepatocellular hypertrophy was also detected only in the 1000 mg/kg/day group (5/10) females. Minimal thyroid follicular cell hypertrophy was detected 1/10, 1/10, 5/10 and 7/10 males in the control, 100, 300 and 1000 mg/kg/day groups, respectively and in 4/10 and 3/10 females in the 300 and 1000 mg/kg/day groups respectively. In addition, mild thyroid follicular hypertrophy was detected in 4/10 females in the 1000 mg/kg/day group. The histologic changes in the liver were accompanied by an increase in liver weight. In contrast there were no statistically significant changes in thyroid weight (absolute, relative to body weight and relative to brain weight). At study week 13, mean liver weights in all dose levels of both sexes (absolute, relative to body weight and relative to brain weight) were increased compared to the male and female control means ($p<0.05$). The increases in liver weight were a result of a microsomal enzyme inducing effect1 and were not typically considered indicative of toxicity in absence of frank organ damage. The reversible histologic changes (vacuolation and hypertrophy) are often found to accompany increased liver weight caused by liver enzyme induction. At week 17, the liver changes (weight and histology) had at least partially, if not fully, resolved in all treated groups without delayed or long-term toxic effects. The histologic changes in the thyroid had also nearly completely resolved except in the 1000 mg/kg/day group females, where partial recovery occurred.

Increases in mean prostate weight were noted in the 1000 mg/kg/day group males at the primary necropsy. However, the increases in prostate weight were probably not of toxicological significance since the increases did not persist to the recovery period, there were no correlating histologic findings and no change in sperm production.

HBCD was detected in the adipose tissue of male and female rats treated with 1000 mg/kg/day for up to 90 days. Isomer-specific analysis showed that the relative isomer concentrations in adipose tissue at all time points were alpha>>gamma>beta which is in contrast to the test article composition (gamma>>alpha>beta). Steady state levels were achieved by study day 27. Levels in male and female rats were similar at all time points and declined during the recovery period.

All the test article-related changes at 100 and 300 mg/kg/day were mild, reversible, generally secondary to hepatic enzyme induction (which is an adaptive not a toxic change) and without effect on the clinical condition of the animals. The additional findings observed at 1000 mg/kg/day (increased gamma glutamyltransferase and additional increases in the size of the liver and prostate), were also reversible, not associated with specific target organ damage or diminished function and were, therefore, probably of limited, if any, toxicologic significance. On this basis the no-observed-adverse-effect level (NOAEL) of HBCD administered to Crl:CD®(SD)IGS BR rats by gavage in corn oil for 90 days is 1000 mg/kg/day.

Conclusions

Input optional information or further explain the contents of a particular section, much as is done in the "Discussion" portion of a publication in academic journals.

The no-observed-adverse-effect level (NOAEL) of HBCD administered to Crl:CD®(SD)IGS BR rats by gavage in corn oil for 90 days is 1000 mg/kg/day, the highest dose tested.

Data Quality

Reliability High

Denote the reliability of data, at the discretion of the person preparing the robust su

Data Reliability Remarks **Add comments about how reliability of data was determined, or add re**

This study was perfomed according to current guideline under good laboratory practices by laboratory with considerable experience in this area.

Reference

Cite the full reference for the critical study on which the robust study summary is based. List other appropriate references that support this summary and, have been reviewed for

>> Remarks

Chengelis, C. An Oral (Gavage) 90 Day Toxicity Study of HBCD in Rats. Laboratory Study No. WIL-186012. WIL Research Laboratories, Inc., Ashland, Ohio. 2001.

General

Add any statement that doesn't fit into any of the other f

Sponsored by the American Chemistry Council's Brominated Flame Retardant Industry Panel (BFRIP).

Sponsor ID	[illegible]	Albemarle Corporation		Create Date	[illegible]6/01
CAS Number	[illegible]	Cyclododecane, 1,2,5,6,9,10-hexabromo-		Study Number	[illegible]
Consortia ID	[illegible]	CMA Brominated Flame Retardant Industry Panel (BFRIP)		Completed	[illegible]

[illegible]

Revision Date: 12/5/01

Test Substance

Remarks: The test article was a commercial HBCD product ("Hexabromid S") produced at one time by BASF in Germany. BASF no longer manufactures HBCD.

Chemical Category

Method

>> Method/Guideline followed

Not specified.

>> GLP Unknown

>> Year study performed 1969

Were Good Laboratory Practices followed in the st

>> Species

rat

>> Strain Mammal strai Sprague-Dawley

>> Sex Both

>> Number of males per dose 10 **>> Number of females per dose** 10

>> Route of Administration Oral

>> Exposure Period 28

Duration of study in days (for example, 28 days, 90 d

>> Frequency of treatment Daily

Number of doses per day, week, etc. This is particularly relevant for inhalation experiments -- 6hrs/day, 5 d

>>Doses 0, 1, 2.5, 5% of the diet.

List all doses used in te

>> Control Group Yes

Concurrent contro

>> Post observation period None.

Length of time animals observed after last d

>> Statistical Method See Remarks.

Cite statistical methods use

Remarks for Method

HBCD ("Hexabromid S") was tested in Sprague-Dawley rats (10/sex/group) at doses of 0, 1, 2.5 and 5% of the diet for 28 days. Doses calculated from the actual body weights and food consumption in this study are 0, 940, 2410, and 4820 mg/kg body weight/day.

Results

>> NOAEL Precision =

>> NOAEL dose 1000 **>> Unit** mg/kg in feed

>> NOAEL Effec

(e.g., decrease in body weight, organ

Increase in liver weight in the absence of pathology and clinical chemistry changes.

>> LOAEL Precision =

>> LOAEL dose 5000 **>> Unit** mg/kg in feed

>> LOAEL Effect (e.g., decrease in body weight, organ Decrease in boudy weight at a dose level of 5% in the diet.

>> Actual dose received by dose level by sex (if availabl

0, 940, 2410, and 4820 mg/kg body weight/day

>> Toxic response A brief narrative describing toxic response or effects, by dos

See Results Remarks.

>> Statistical results Note statistical results, with appropri ρ value

See Results Remarks.

Results Remark Provide at a minimum qualitative descriptions of elements where dose effect related observations of elements were seen:

HBCD ("Hexabromid S") was tested in Sprague-Dawley rats (10/sex/group) at doses of 0, 1, 2.5 and 5% of the diet for 28 days. Doses calculated from the body weights and food consumption are 0, 940, 2410, and 4820 mg/kg body weight/day.

No clinical signs were observed at the 1% dose levels. No significant change in mean body weight between the control and the 1 and 2.5% dose levels. The mean liver weights (absolute and relative to body weight) were different from the control mean (increased) at all dose levels, but no microscopic pathology was detected. Thyroid hyperplasia was reported in some animals at all doses, as was "very slight numerical development of the follicles and ripening follicles in the ovaries of females" at the high dose (4820 mg/kg/d). No gross or microscopic changes were detected in any other organ, and no change was detected in clinical chemistry tests.

The report concluded that "The increased liver weight must be attributed to hyperactivity; hypermetabolism as a result of increased thyroid activity appears probable in view of the observations of the thyroid". Therefore, the increased liver weights were not pathologic: there

were no microscopic lesions detected on histopathology and no change in clinical chemistry values. Recent work on the relationship of liver weight, microsomal enzyme induction, and histological change in rat toxicology studies has been published (Amacher et al, Food and Chemical Toxicology, 36, 831-839, 1998). This paper concluded "The preponderance of data collected in these 11 studies indicates that microsomal enzyme induction was not accompanied by evidence of chemically-induced liver injury. We conclude that in the rat, both hepatomegaly and microsomal enzyme induction are benign and adaptive changes in response to certain chemicals that stimulate the hepatic drug metabolizing enzyme system."

Conclusions

Input optional information or further explain the contents of a particular section, much as is done in the "Discussion" portion of a publication in academic journals.

The NOAEL in this 28-day study was 1% "Hexabromid S" in the diet. Based on body weights and food consumption data this dose is equivalent to 940 mg/kg body weight/day.

Data Quality

Reliability Reasonable

Denote the reliability of data, at the discretion of the person preparing the robust su

Data Reliability Remarks **Add comments about how reliability of data was determined, or add re**

This study was performed by a laboratory with considerable experience. However, the study was performed approximately 30 years ago using an HBCD product no longer manufactured as test article, and is not up to today's standards. The fact that recently conducted repeated dose studies with HBCD provided comparable results lends credence to the results of this study.

Reference

Cite the full reference for the critical study on which the robust study summary is based. List other appropriate references that support this summary and, have been reviewed for

>> Remarks

Zeller H and Kirsch P (1969) Hexabromocyclododecane: 28-day feeding trials with rats. BASF (unpublished laboratory report).

General

Add any statement that doesn't fit into any of the other f

This study was sponsored and performed by BASF.

The doses and results of this study are improperly reported by the Swedish Chemicals Inspectorate KEMI in the 1999 draft EU risk assessment of HBCD and in reports to the OECD SIDS programme. KEMI reports the doses as 0, 500, 1250 and 2500 mg/kg body weight/day (basis for conversion not given), and that the low-adverse-effect-level was 500 mg/kg (the 1% in the diet dose).

Sponsor ID	[redacted]	Albemarle Corporation		Create Date	[redacted]
CAS Number	[redacted]	Cyclododecane 1,2,5,6,9 10-hexabromo-		Study Number	[redacted]
Consortia ID	[redacted]	CMA Brominated Flame Retardant Industry Panel (BFRIP)		Completed	[redacted]

[redacted]

Revision Date: 12/6/01

Test Substance

Remarks: The test article was a commercial HBCD product ("Hexabromid S") produced at one time by BASF in Germany. BASF no longer manufactures HBCD.

Chemical Category

Method

>> Method/Guideline followed

Not known.

>> GLP Unknown

>> Year study performed 1970

Were Good Laboratory Practices followed in the st

>> Species

rat

>> Strain Mammal strai Sprague-Dawley

>> Sex Both

>> Number of males per dose 20 **>> Number of females per dose** 20

>> Route of Administration Oral

>> Exposure Period 90

Duration of study in days (for example, 28 days, 90 d

>> Frequency of treatment Daily

Number of doses per day, week, etc. This is particularly relevant for inhalation experiments -- 6hrs/day, 5 d

>>Doses 0, 0.16, 0.32, 0.64 and 1.28% of the diet

List all doses used in te

>> Control Group Yes

Concurrent contro

>> Post observation period 42 days.

Length of time animals observed after last d

>> Statistical Method See Remarks.

Cite statistical methods use

Remarks for Method

HBCD ("Hexabromid S") was tested in Sprague-Dawley rats at doses of 0, 0.16, 0.32, 0.64 and 1.28% of the diet for 90 days. Doses calculated on the actual body weights and food consumption in this study reveals: 0, 120, 240, 470 and 950 mg/kg body weight/day.

Results

>> NOAEL Precision =

>> NOAEL dose 1280 **>> Unit** mg/kg in feed

>> NOAEL Effec

(e.g., decrease in body weight, organ

Increase in liver weight in the absence of pathology or clinical chemistry changes.

>> LOAEL Precision >

>> LOAEL dose 1280 **>> Unit** mg/kg in feed

>> LOAEL Effect

(e.g., decrease in body weight, organ

See Remarks.

>> Actual dose received by dose level by sex (if availabl

0, 120, 240, 470 and 950 mg/kg body weight/day

>> Toxic response A brief narrative describing toxic response or effects, by dos

See Remarks.

>> Statistical results Note statistical results, with appropri ρ value

See Results Remarks.

Results Remark Provide at a minimum qualitative descriptions of elements where dose effect related observations of elements were seen:

HBCD ("Hexabromid S") was tested in Sprague-Dawley rats at doses of 0, 0.16, 0.32, 0.64 and 1.28% of the diet for 90 days. Doses calculated on the actual body weights and food consumption in this study reveals: 0, 120, 240, 470 and 950 mg/kg body weight/day.

Doses up to 0.64% (470 mg/kg/d) produced no adverse clinical signs, no change in body weight, and no change in clinical chemistry results. An increase in the relative liver to body weight ratio was found, and was accompanied by fatty accumulation but no other histologically discernible changes were detected in the liver. The pathology report states that although fat ("lipid phanerosis") was visible microscopically in the liver of treated rats, this change was not accompanied by any pathology and could not be defined as "fatty liver". Further, no histological changes were found in any other organ. The report states that in the "absence of detectable clinico-chemical disturbances or histological changes of the vital organs, it was concluded that

the increased liver weight and the fat deposits, both of which were largely reversible when administration of Hexabromid S was stopped, were the result of a temporary increase in the activity of the liver."

Conclusions

Input optional information or further explain the contents of a particular section, much as is done in the "Discussion" portion of a publication in academic journals.

The highest dose tested in the BASF 90 d study, 1.28% of the diet (950 mg/kg body weight/day), is the no adverse effect level or NOAEL.

Data Quality

Reliability Reasonable

Denote the reliability of data, at the discretion of the person preparing the robust su

Data Reliability Remarks **Add comments about how reliability of data was determined, or add re**

This study was performed by a laboratory with considerable experience. However, the study was performed approximately 30 years ago using an HBCD product no longer manufactured as test article, and is not up to today's standards. The fact that recently conducted repeated dose studies with HBCD provided comparable results lends credence to the results of this study.

Reference

Cite the full reference for the critical study on which the robust study summary is based. List other appropriate references that support this summary and, have been reviewed for

>> Remarks

Zeller H and Kirsch P (1970) Hexabromocyclododecane: 90-day feeding trials with rats. BASF (unpublished laboratory report).

General

Add any statement that doesn't fit into any of the other f

This study was sponsored and performed by BASF.

The Swedish Chemicals Inspectorate (KEMI) improperly reported the results of this study and incorrectly converted the doses from a percentage of the diet to mg/kg body weight in 1999 draft EU risk assessment on HBCD and in reports to the OECD SIDS programme. KEMI converted the dietary doses to 0, 80, 160, 320 and 640 mg/kg body weight/day (basis for conversion not given). KEMI also stated that the low-adverse-effect level (LOAEL) in this

study was 80 mg/kg (the 0.16% in the diet dose level, and that a no effect level (NOEL) was not determined in any of the subchronic studies conducted to date, including the 1997 Rat 28-Day Study.

The results of the BASF 90 day study do not indicate that an adverse effect was produced at 0.16% of the diet. Further, the results indicate no adverse effect was produced at the highest dose tested, 1.28% of the diet. The BASF pathology report clearly states that although fat ("lipid phanerosis") was visible microscopically in the liver of treated rats, this change was not accompanied by any pathology and could not be defined as "fatty liver". Therefore, not even the highest dose tested in the 90-day study can be defined as the low adverse effect level (LOAEL). The highest dose tested in the BASF 90 d study, 1.28%, is more accurately defined as the no adverse effect level or NOAEL.

EPA High Production Volume (HPV) Track

Toxicity End point:
Toxicity in Vitro (Gene Mutations)

Sponsor ID	1100021	Albemarle Corporation	Create Date	1/6/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y

Revision Date: 12/5/01

Test Substance

Remarks: The test article was a composite of equal parts of the commercial hexabromocyclododecane (HBCD) commercial product produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. The test article composite was analyzed for characterization and homogeneity. The results of the analysis indicated the test article was homogeneous and contained the following components: HBCD beta isomer 8.5%, HBCD alpha isomer 6.0%, HBCD gamma isomer 79.1%.

Chemical Category

Method

>> Method/Guideline followed

EPA OPPTS Method 870.5375 In vitro Mammalian Chromosome Aberration Test

>> Test Type

Cytogenetic assay

>> System of Testing: Non-bacterial

>> GLP: Yes

>> Year study performed: 1996

>> Species

Primary cultures - human lymphocytes

>> Metabolic Activation

Arochlor 1254-induced rat liver S-9; prepared from male Sprague-Dawley rats

>> Concentration

Initial: 75, 250, 750, 2500 ug/ml; Definitive: 10, 19, 38, 75, 150, 300, 600 ug/ml

>> Statistical Method: Fisher's exact test

Remarks for Method

The test article, Hexabromocyclododecane (HBCD) was tested in the in vitro mammalian cytogenetic test using human peripheral blood lymphocytes (HPBL) in both the absence and presence of metabolic activation. The assay was performed in two phases. The first phase, the initial chromosome aberration assay, was conducted to establish the dose range for testing and to evaluate the clastogenic potential of the test article. The second phase, the independent repeat chromosome aberration assay, was performed to confirm the test system response to the test article seen in the initial assay.

Dimethylsulfoxide (DMSO) was the solvent of choice based on the solubility of the test article and compatability with the target cells. The test article was soluble in DMSO at ~500 mg/ml, the highest concentration tested.

Intial. In the initial chromosome aberration assay, duplicate cultures of HPBL were exposed to 9 concentrations of the test article, and to positive, solvent and negative controls. The dividing cells were harvested at ~20 hours after initiation of treatment. The maximum dose tested was 2500 ug/ml. Dose levels greater than 2500 ug/ml were insoluble in the treatment medum and not tested. Visible precipitate was observed in treatment medium at dose levels of 750 and 2500 ug/ml and was soluble but cloudy (no visible precipitate) at dose levels 75 and 250 ug/ml. The test article was soluble in treatment medium at all other dose levels tested. In the non-activated portion of the test, HPBL cells were exposed to the test article continuously for 20 hours; in the S9-activated portion of the test, HPBL were exposed to the test article for 4 hours. Metaphase cells were collected for microscopic evaluation at 20 hours after the initiation of treatment.

Second Phase. Duplicate cultures of HPBL were exposed to at least 4 concentrations of the test article, as well as solvent, positive, and untreated controls. The dose levels selected were based on the initial assay. The dividing cells were harvested at 2 time points: 20 and 44 hours after initiation of treament. HBCD was tested in the absence and presence of an Arochlor-induced S9 metabolic activation system at dose levels of 10, 19, 38, 75, 150, 300 and 600 ug/ml. The test article was soluble but cloudy at 75 ug/ml and was workable in treatment medium at dose levels 150 ug/ml and higher. The test article was soluble in treatment medium at all other concentrations tested. In the independent repeat assay, HPBL cells were exposed to the test article continuously for 20 or 44 hours in the non-activated test system and for 4 hours in the S9-activated test system. Metaphase cells were collected for microscopic evaluation in both the non-activated and S9-activated studies at 20 and 44 hours after the initiaiton of treatment.

Evaluation of Metaphase Cells. Metaphase cells with 46 centromeres were examined under oil immersion without knowledge of treatment groups. Whenever possible, a minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined and scored for chromatid-type and chromosome-type aberrations. The mitotic index was recorded as the percentage of cells in mitosis per 500 cells counted. In the delayed harvests, the percent polyploid cells was recorded per 100 metaphase cells.

Controls. Mitomycin C was used as the positive control in the non-activated study. Cyclosphosphamide was used as the positive control in the S-9 activated study. For both positive controls one dose with sufficient scorable metaphase cells was selected for analysis. The solvent vehicle for the test article was used as the solvent control at the same concentration as that found in the test article-treated groups. Growth medium or S9 reaction mixture was used in the untreated control.

Evaluation of Results. Toxic effects of treatment were based on mitotic inhibition relative to the solvent-treated control. The number and types of aberrations, the percent aberrant cells, the percentage of numerically damaged cells and the frequency of structural aberrations per cell

was reported for each treatment group.

Results

>> Result Negative

>> Cytotoxic Concentration

Non-activated: toxicity at 750 ug/ml; S9-activated: toxicity at 250 ug/ml

>> Genotoxic Effects Unconfirmed

>> Statistical results

No statistically significant differences were observed between the negative, solvent and treatment groups ($p>0.05$, Fisher's exact test). The positive controls performed as expected.

Results Remark

In the initial assay, dose levels of 2500 ug/ml in the non-activated study and 750 and 2500 ug/ml in the S9-activated study were not analyzed from chromosome aberrations due to complete mitotic inhibition. Toxicity (mitotic inhibition) of ~56% was observed at the highest dose level (750 ug/ml) evaluated for chromosome aberrations, in the non-activated study. In the S9-activated study, 13% toxicity was observed at the highest dose level (250 ug/ml) evaluated for chromosome aberrations. No statistically significant increases in chromosome aberrations were observed in either the non-activatged or S9-activated test systems relative to the solvent control group regardless of dose level ($p>0.05$, Fisher's exact test).

In the independent repeat chromosome aberration assay, toxicity, as measured by mitotic inhibition, was ~55% and 94% at the 20 and 44 hour harvest, respectively, at the highest dose levels (600 and 300 ug/ml) evaluated in the non-activated studies. In the S9-activated studies, toxicity was approximately 71% and 69% at the 20 and 44 hour harvest, respectively, at the highest dose levels (300 and 600 ug/ml) evaluated. The 600 ug/ml dose level in the non-activated 44 hour harvest and in the S9-activated 20 hour harvest was not analyzed for chromosome aberrations due to an insufficient number of scorable metaphase cells. No statistically significant increases in structural chromosome aberrations were observed in either the non-activated or S9-activated studies, regardless of dose level or harvest time ($p>0.05$, Fisher's exact test). No statistically significant increases in numerical chromosome aberrations were observed in either the non-activated or S9-activated studies at the 44 hour harvest time, regardless of dose level ($p>0.05$, Fisher's exact test).

Conclusions

EPA High Production Volume (HPV) Track

Toxicity End point:
Toxicity in Vitro (Gene Mutations)

Sponsor ID	110021	Albemarle Corporation		Create Date	1/6-01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-		Study Number	1
Consortia ID	101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)		Completed:	Y

HBCD was negative for the induction of structural and numerical chromosome aberrations in human peripheral blood lymphocytes.

Data Quality

Reliability High

Data Reliability Remarks

This study was performed using current techniques, under Good Laboratory Practices, by a laboratory with considerable experience performing this type of study.

Reference

>> Remarks

Gudi, R. And Schadly, E. (1996) Chromosome Aberrations in Human Peripheral Blood Lymphocytes. Hexabromocyclododecane. Laboratory Study Number G96AO61.342. Microbiological Associates, Inc., Rockville, MD.

General

Study sponsored by the Chemical Manufacturers Association Brominated Flame Retardant Industry Panel.

EPA High Production Volume (HPV) Track

Toxicity End point:
Toxicity In Vitro (Gene Mutations)

Sponsor ID	110021	Albemarle Corporation	Create Date	1/1/01	
CAS Number	3194-55-6	Cyclododecane, 1,2,5,6,9,10-hexabromo	Study Number	2	
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y	

Revision Date: 12/5/01

Test Substance

Remarks Exact composition of the test article is not known.

Chemical Category

Method

>> Method/Guideline followed

Not specified

>> Test Type

Ames test

>> System of Testing Bacterial

>> GLP Unknown

>> Year study performed 1976

>> Species

Salmonella typhimurium

>> Metabolic Activation

Arochlor induced rat liver S9

>> Concentration

0, 1, 10, 50, 100, 500, 1000, 5000 ug/plate

>> Statistical Method Not known.

Remarks for Method

Five strains of Salmonella typhimurium (TA1535, TA1537, TA1538, TA98 and TA100) were tested in the presence and absence of a metabolic activation system (Arochlor induced rat liver). Doses were 0, 1, 10, 50, 100, 500, 1000 or 5000 ug HBCD/plate.

EPA High Production Volume (HPV) Track

Toxicity End point:
Toxicity In Vitro (Gene Mutations)

Sponsor ID	[redacted]	Albemarle Corporation	Create Date	[redacted]	
CAS Number	[redacted]	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	[redacted]	
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y	

Results

>> Result Negative

>> Cytotoxic Concentration

>5000 ug HBCD/plate with or without metabolic activation

>> Genotoxic Effects Unconfirmed

>> Statistical results

Not known.

Results Remark

The test article was not mutagenic or toxic at any dose level when tested with or without metabolic activation.

Conclusions

HBCD was not mutagenic in S. typhimurium at doses up to and including 5000 ug/plate when tested with or without metabolic activation. These results are consistent with other Ames test's performed on this material (Ogaswara S and Hanafusa T. (1993) Report on mutagenicity test on Pyroguard SR-103 using microorganisms; Baskin A and Phillips, B. (1977) Mutagenicity of two lots of FM-100, Lot 53 and residue of Lot 3322 in the absence and presence of metabolic activation. Industrial Biotest Laboratories, Sponsored by Velsicol Chemical Corporation; Anonymous. (1979) Mutagenicity test of GLS-S6-41A. Gulf South Research Institute, Sponsored by Ethyl Corporation; US Environmental Protection Agency (1990) Ames metabolic activation test to assess the potential mutagenic effect of Compound No. 49. Letter from BASF. EPA/OTS Doc #86-900000385.

Data Quality

Reliability Acceptable

Data Reliability Remarks

Multiple Ames tests performed at different test laboratories using different commercial HBCD products as test article have all been negative. The consistency of the negative results increases the confidence in the results.

EPA High Production Volume (HPV) Track

Toxicity End point:
Toxicity In Vitro (Gene Mutations)

Sponsor ID	1200021	Albemarle Corporation	Create Date	4/6/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	2
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y

Reference

>> Remarks

Simmons V., Poole, D., Newell, G., and Skinner, W. (1976) In vitro microbiological mutagenicity studies for four CIBA-GEIGY Corporation compounds. SRI Project LSC-5702.

General

EPA High Production Volume (HPV) Track

Toxicity End point:
Toxicity In Vitro (Gene Mutations)

Sponsor ID	[illegible]	Albemarle Corporation	Create Date	[illegible]	
CAS Number	[illegible]	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	[illegible]	
Consortia ID	110012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y	

Revision Date: 12/6/01

Test Substance

Remarks: HBCD, obtained from Aldrich Chemicals (Stockholm, Sweden)

Chemical Category

Method

>> Method/Guideline followed

Non standard test methodology

>> Test Type

Mammalian cells in culture (Sp5 and SPD8 duplication cell lines)

>> System of Testing: Non-bacterial

>> GLP: No

>> Year study performed: 1999

>> Species

Not known.

>> Metabolic Activation

None

>> Concentration

See results.

>> Statistical Method: Student's t test

Remarks for Method

HBCD was tested in vitro in hamster cells (Sp5/V79 and SPD8) in a recombination assay at five doses between 2 and 20 ug/ml plus a control. The Sp5 and SPD8 clones exhibit a spontaneous partial duplication of the HPRT gene, resulting in a non-functional HGPRT protein. The mutants revert spontaneously to a functional HPRT gene phenotype by recombination; an increase in reversion frequency is considered a positive response.

Treatment with HBCD resulted in a ~ maximal 2-fold increase in revertant frequency, which

was reported as statistically significant.

This reliability of this genetic test is unknown. The reproducibility of the results, validation of the system, dose-effect response, and whether a maximal two-fold increase is evidence of a positive response are also unknown.

Results

>> Result Ambiguous

>> Cytotoxic Concentration

Not known.

>> Genotoxic Effects Equivocal

>> Statistical results

See Remarks Secttion.

Results Remark

Treatment with HBCD resulted in a ~ maximal 2-fold increase in revertant frequency, which was reported as statistically significant.

This reliability of this genetic test is unknown. The reproducibility of the results, validation of the system, dose-effect response, and whether a maximal two-fold increase is evidence of a positive response are also unknown.

Conclusions

Treatment with HBCD resulted in a ~ maximal 2-fold increase in revertant frequency, which was reported as statistically significant. The reliability of this test is unknown.

Data Quality

Reliability Unknown.

Data Reliability Remarks

Sponsor ID	1100021	Albemarle Corporation	Create Date	4/6/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	3
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y

The reliability and predictive ability of this genetic test is unknown. The reproducibility of the results, validation of the system, dose-effect response, and whether a maximal two-fold increase is evidence of a positive response are unknown.

Reference

>> Remarks

Helleday et al, Mutat Res, 1999, 439(2): 137-147.

General

EPA High Production Volume (HPV) Track

Toxicity End Point:
Toxicity In Vivo (Chromosomal Aberrations)

Sponsor ID	1000021	Albemarle Corporation	Create Date	1/1/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y

Revision Date: 12/5/01

Test Substance

Remarks: The test article was a composite of equal parts of the commercial hexabromocyclododecane (HBCD) commercial product produced by Albemarle Corporation, Dead Sea Bromine Group, and Great Lakes Chemical Corporation. The test article composite was analyzed for characterization and homogeneity. The results of the analysis indicated the test article was homogeneous and contained the following components: HBCD beta isomer 8.5%, HBCD alpha isomer 6.0%, HBCD gamma isomer 79.1%.

Chemical Category

Method

>> Method/Guideline followed

OECD Method 474

>> Test Type

Micronucleus assay

>> GLP: Yes

>> Year study performed: 2000

>> Species

mouse

>> Strain: **Mammal strai:** NMRI

>> Sex: M

>> Number of males per dose: 5 **>> Number of females per dose:** 0

>> Route of Administration

Intrperioneal

>> Doses: 0, 500, 1000, 2000 mg/kg

>> Exposure period: Two doses administered 24 hrs apart.

>> Statistical Method: Wilcoxon test

Remarks for Method

Sponsor ID	110002	Albemarle Corporation	Create Date	1/6/01
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1
Consortia ID	101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y

Hexabromocyclododecane (HBCD) was tested for clastogenicity and for the ability to induce spindle poison effects in NMRI mice (Charles River Deutschland GmbH) using the micronucleus method. HBCD, dissolved in DMSO, was administered twice intraperitoneally with a 24-hr interval between doses to male mice (n=5/group) at dose levels of 500, 1000 or 2000 mg/kg body weight in a volume of 4 ml/kg. DMSO (the vehicle) was administered to male mice by the same route and frequency. Cyclophosphamide was used as a positive control for clastogenic effects. Vincristine was used as a positive control for induction of spindle poison effects. Animals in the positive control groups were treated only once.

The animals were sacrificed and the bone marrow of the two femora prepared 24 hours after the second administration. After staining, 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes with and without micronuclei occurring per 2000 polychromatic erythrocytes were also counted.

Results

>> Effects on Mitosi

PCE/NCE 0, 500, 1000, 2000 mg/kg = 3.74, 2.89, 2.67, 2.49, respectively.

>> Genotoxic Effects Negative

>> Statistical results

No statistical differences between the treatment and vehicle control group were observed (p<=0.05).

Results Remark

The two intraperitoneal administrations of DMSO in a volume of 4 ml/kg body weight led to 1.4% polychromatic erythrocytes containing micronulei. In the 2000 mg HBCD/kg body weight group, 0.9% micronuclei were found. In the 1000 and 500 mg HBCD/kg body weight groups, 1.0 and 1.1% micronuclei were detected. The two positive control substances performed as expected.

The number of normochromatic erythrocytes containing micronuclei did not diffeer to any appreciable extent in the negative control or various dose groups.

Conclusions

HBCD treatment did not increase numbers of micronuclei. The number of normochromatic or polychromatic erythrocytes containing small micronuclei did not deviate from the vehicle control value and was within the historical control range. Large micronuclei were not observed. HBCD had no chromosome-damaging (clastogenic) effect in this study and did not impair chromosome distribution during mitosis.

EPA High Production Volume (HPV) Track

Toxicity End Point:
Toxicity In Vivo (Chromosomal Aberrations)

Sponsor ID	1300021	Albemarle Corporation	Create Date	12/20/01	
CAS Number	3194556	Cyclododecane, 1,2,5,6,9,10-hexabromo-	Study Number	1	
Consortia ID	1101012	CMA Brominated Flame Retardant Industry Panel (BFRIP)	Completed:	Y	

Data Quality

Reliability High

Data Reliability Remarks

This study was performed according to current guidelines under Good Laboratory Practices by an experienced laboratory.

Reference

>> Remarks

Engelhardt, G and Hoffmann, H. (2000) Cytogenetic Study in vivo with Hexabromocyclododecane in the Mouse Micronucleus Test After Two Intraperitoneal Adminstrations. Laboratory Project Identification: 26M0100/004018. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen, Germany.

General